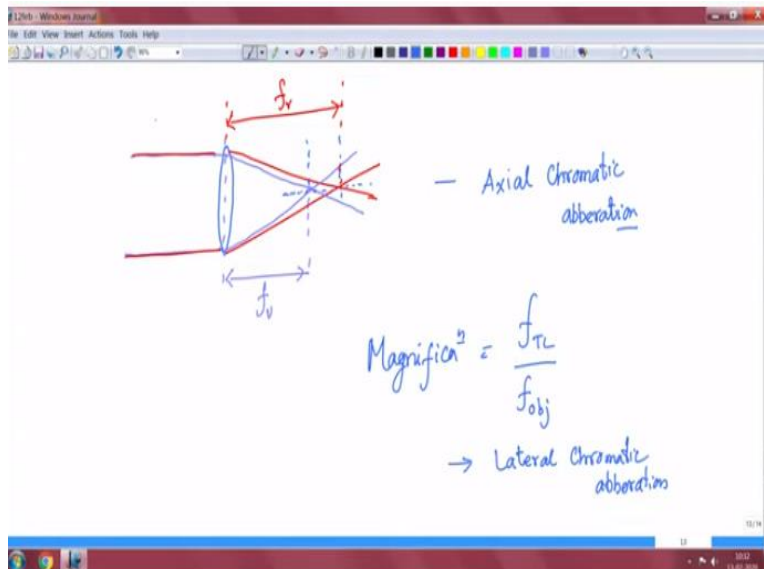


Optical Spectroscopy and Microscopy
Prof. Balaji Jayaprakash
Department: Centre for Neuroscience
Indian Institute of Science – Bangalore

Lecture – 52
Fundamentals of Optical Measurements and Instrumentation

Hello and welcome to this course on optical spectroscopy and microscopy in the last lectures we were actually talking about the objective lenses and we saw some of the real-world objective lenses how they look and we looked at the individual parameters that were listed on the objective lens and what they mean in that line we were focusing on one of the aberrations chromatic aberrations just to rehash when we have a lens and now we can go to the whiteboard.

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So when we have a lens and if you try to focus the light through the lens the focal length of, I mean the blue light, or the violet light and the red light are different by the virtue of the refractive index of these lights being different right. So we were talking about the speed of the speed dependent mean the speed being dependent on the wavelength I mean the color of the light that we choose here and then as a result, the refractive index being different.

As a result, I can I said that okay the refractive index you can think of as the ability to refract right. So that is being different so the focus itself is different and since the focus itself is different, so we have 2 effects one is that the place at which the light come into focus right. So

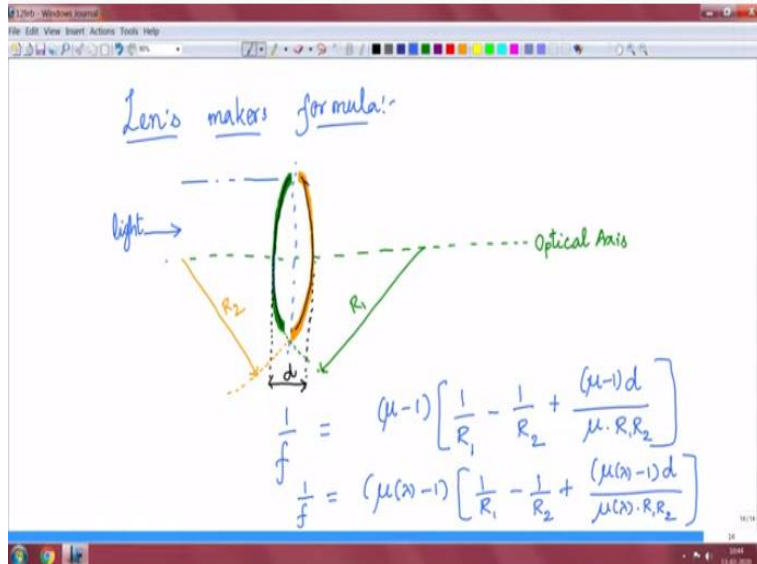
will be different so we call that as axial chromatic aberration because that is represented by the fact that we are looking at the cross-section and then you have 2 points in the axis of propagation on which the lights comes into focus.

So we call that as f_v and f_r and so since these 2 focus are different you have the axial chromatic aberrations. So if you take a microscope and look at it under the microscope there is a axial chromatic aberration what you would see is that the colored object alright will have different will come to focus in different focal planes depending on the wavelength okay. So this in a one chroma for a situation you might be able to it be hard for you to actually visualize with your eye.

However it has an important very important impact if you have to make use of co-localization studies or if you are talking about or measuring where exactly this sort of force are present depending on the bandwidth of it you might see them, they may be coming at different I mean they will be coming to focus at different planes. And along with that what you will see is that that since the focal length of these 2 colors are different the magnification per say as seen by the microscope for this violet and the red light will also be different.

Now that results in lateral chromatic aberration. So when you look at an object then you will see there are only its aged you have at this dispersion of different colors. Before that all along the sample plane when there are objects present inside of that you may not be able to see it because it just that the overlap of different wavelengths and there you may not be able to spot that difference. However if you look around the edge then if you see different colors of forming shadows so to speak or Hallos around the sample then you know it is there is a chromatic aberration.

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So now we can look at this a little bit more formally. So where is this dependence coming from, the dependence coming from the fact that focal length of the lens itself is dependent on the wavelength. The wavelength I mean of the light so to understand this let us look at what is commonly referred as lens maker's formula or an equation again very simple geometric optics. So which we have probably all have learnt at the school level.

But we know that if you have our lens in this case, I am actually taking a bi convex lens the generalization is simple we may be able to okay maybe we will what we will go through that both the generic surfaces. So let us take a bi convex lens and we want to know when what is the refracting power of this lens right what is how well the light going through this lens is going to refract right.

So how much of that is going to happen so now that we define by asking how far off from the central plane of this lens do, we see that ray is coming into focus. So what we are actually expecting is that we are measuring this distance f now this f from the lens maker's formula you can actually write down as actually you write down $1/f$ is given by $n - 1$ - where n actually we are talking about μ .

So let us stick to μ , μ being the refractive index - 1 and divide by $1/R_1 - 1/R_2 + \mu - 1 d / \mu R_1 R_2$ alright. So just using simple geometric optics we would be able we should be able to

get this in fact many of the textbooks would show you how to actually obtain this expression, but the point here is that how can we use this to understand chromatic aberration and then probably propose a solution for correcting that right.

I mean people do use I mean the objective lens that I showed you it clearly saw that there are many I mean it is pretty thick object and it has many elements inside it. So how are they doing this correction right, so they are doing it at multiple levels too. So we will try to understand this that using this equation here all right. So now what are this R_1 R_2 d and so on. R_1 is the radius of curvature of the first surface.

Now what is the first surface so there is a convention to it many of you may be already familiar. But the convention is the convention that I am using here is that I am going along the direction of the incident light. So that your light rays shown on to the lens along this in this direction. So when it when it happens in this direction. So then the first surface that the light encounters we call that as this first I mean the radius of curvature of that surface we call it as R_1 .

So in this case the radius of curvature of the first surface is this blue. So I am going to color it, or I am going to highlight that with dark green okay now and then of course extend that right. So the you can see that this radius of curvature it is a ; it is like a circle and it acts I said that it is extending on to this object. For the time being let us to reduce the confusion we will just only constant draw the optical axis here right.

So that is our optical axis and here this is our R_1 . Now clearly then the second surface its a bi convex lens the second surface then let us highlight with bright orange and then extend it right and second surface here and extend. Now that is acting as if that is my R_2 the radius of curvature of the second surface and its important to note there are sign conventions which is where are we actually measuring these surfaces from.

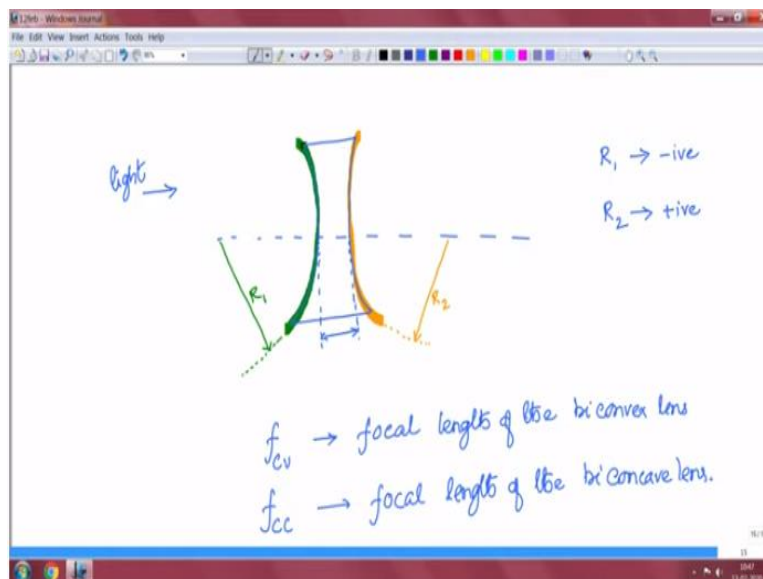
So that convention I mean the in this formula what we are going to make an assumption of is the sign with reference to a particular surface well its a particular plane. So let us take the central plane we would be as taking that as 0. So everything on to the right of it we are going to measure

it in we are going to call it as positive and everything to the left of it were going to call it as negative all right.

So in that note that is the convention that we follow here. So when we follow then you can actually see R_2 being negative and R_1 would be positive then what is d here. The d itself is the thickness of the lens okay so the thickness of the lens is measured as if you have to draw vertical planes here the separation between these, we call it as d that is the thickness of the lens. Now where is the wavelength dependence here.

Now the wavelength dependence we have to specifically put the refractive index we know is dependent on the wavelength. So, to be specific if we have to write in a complete sense then we have to write it as $1/f = \mu(\lambda) - 1, 1/R_1 - 1/R_2 + \mu(\lambda) - 1 d$ divided by $\mu(\lambda) R_1 R_2$ good. So now clearly now we can see that the f that we calculated will also be a function of the λ itself apart from the radius of curvature and the thickness of the lens and stuff. Given a lens you would still see that the depending on the color. You are going to have different focus okay. So now what we so that is for the bi convex lens that we have drawn here.

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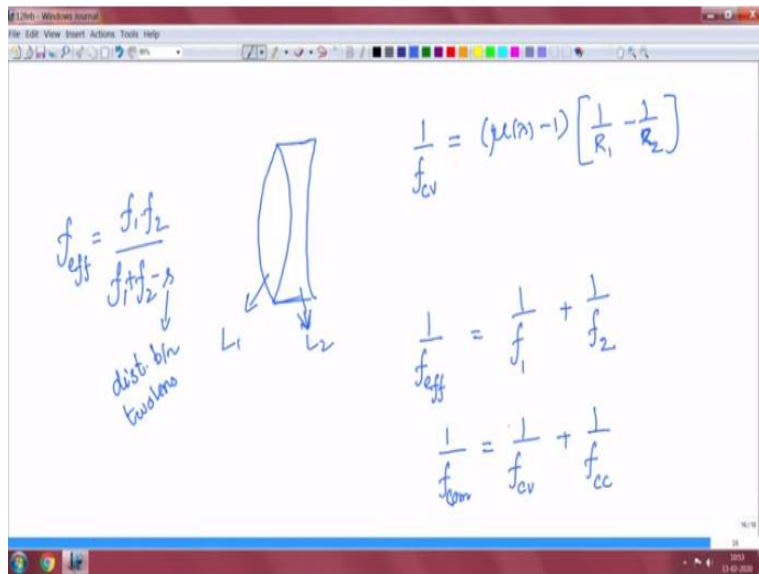
We can do the similar thing for a biconcave lens okay. So we can do the same thing for a concave lens we need to identify the surfaces R_1 and R_2 . So in here the first surface again we are going to identify as that is the first surface that the light faces. So that is our R_1 however now

you see that unlike the previous bi convex lens the R1. So the light is coming from this side right incident the light is incident from the left hand side.

So unlike the bi convex lens the previous one now the R1 that is optical axis R1 is on the left hand side here and R2 is on the right-hand side which means in this case our R1 is negative and R2 actually we should write negative and R2 is positive and the distance d is the thickness of the lens is we need to draw the 2 vertices to the surface and then and we can actually write the same expression the 1 / f and so its, but everything will remain exactly the same except now the r1 and R2 the value that you measure will be changing signs all right.

So now the point here is that now let us call this f as our 1 / f concave convex CV as our focal length. So let us call that the f CV be the focal length of the bi convex lens and f CC be the focal length of the bi concave lens alright. So now what we are going to see now, we know that both of them are function of lambda alright.

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So the claim here is that one of the geometries which can actually get rid of or correct for this chromatic aberration is the combinatorial use of combination of the convex and the concave lens together okay. So the proposition is that if one were to use a convex followed by a Plano concave okay or a biconcave, we will you can use the since we are talking about the bi concave let us use

that and a combination of such lenses can actually take care of the chromatic aberrations that is the proposition.

Now how would you go about designing something like that right. So in order to do that so we first understand that we have to look at so that the general idea here is that we know our the focal length $1/f$ the expression for that right and we also know the if the effective focal length the expression for effective focal length of a lens combination okay. We are going to make an assumption here about these lenses being on thin lens as they obey thin lenses so what is defined as a what would qualify as a thin lens.

If you look at the formula here so the thickness of the lens enters into this equation at this place right the d . So, as long as we satisfy the condition that the d is much smaller than the radius of the curvature of the surfaces then you would say these lenses or thin lens thin lenses. So in such a case what we can actually do is that we can simplify this $1/f$. Let us write down for a concave and if you simplify what is going to happen is that we are going to neglect this whole term.

So what we will have this μ of length $\lambda - 1/R_1 - 1/R_2$ irrespective of whether it is concave or convex is so μ of λ $1/R_1 - 1/R_2$ and the if you have 2 lenses so what we are going to do is we are going to use because since we are actually putting 2 lenses together so it is like we have a lens 1 and then a lens 2 both of them are thin lenses then we can actually write the effective focal length of a lens combination is $1/f$ effective is $f_1 + f_2$ okay the d is just kept next to each other.

So this in such a case its true in general when we have when you have 2 lenses separated by d then you will be probably more familiar with the fact that the effective would be $f_1 f_2$ divided by $f_1 + f_2 - d$ now this d is not the thickness d here etc. Let us rename it as yes this is the distance between the 2 lenses. Since in our case its 0 so one can actually rewrite that expression as $1/f$ effective equal to $1/f_1 + 1/f_2$. Now we do this for this concave and convex which means in our case the combination f combination is going to be 1 okay. So it is a additive expression like this.

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$$\frac{1}{f_{\text{comb}}} = \frac{f_{\text{cc}} + f_{\text{cv}}}{f_{\text{cc}} f_{\text{cv}}}$$

$$\frac{d f_{\text{comb}}}{d \lambda} = \frac{d \left(\frac{f_{\text{cc}} + f_{\text{cv}}}{f_{\text{cc}} f_{\text{cv}}} \right)}{d \lambda} =: 0$$

$$f_{\text{cv}} = \frac{1}{(\mu_{\text{cv}} - 1)} \cdot k_{\text{cv}}, \quad f_{\text{cc}} = \frac{1}{(\mu_{\text{cc}} - 1)} \cdot k_{\text{cc}}$$

$$f_{\text{comb}} = \frac{k_{\text{cc}} \cdot k_{\text{cv}}}{(\mu_{\text{cc}} - 1)(\mu_{\text{cv}} - 1)} \cdot \frac{1}{\frac{k_{\text{cv}}}{(\mu_{\text{cv}} - 1)} + \frac{k_{\text{cc}}}{(\mu_{\text{cc}} - 1)}} = \frac{k_{\text{cc}} \cdot k_{\text{cv}}}{k_{\text{cv}}(\mu_{\text{cc}} - 1) + k_{\text{cc}}(\mu_{\text{cv}} - 1)}$$

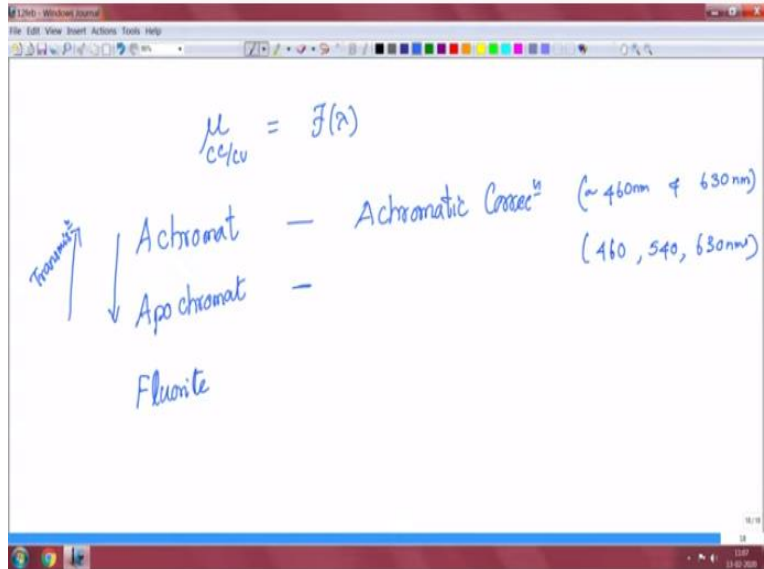
Then we can go ahead and rearrange for is and now we know these are functions of lambda. So what we are actually looking for is we actually are going to write down this expression for f combination and then differentiate that with respect to lambda such that so this expression with respect to lambda and then we want that to be set we want that to be set to 0 right. If you solve for this and we will you can act you can see okay.

So let us quickly do that so as you can see that the equation simplifies to $1 / f_{\text{mu of lambda}} - 1 / R1 - 1 / R2$ given a lens this is a constant. So you could write so $1 / R1 - 1 / R2$ is a constant it does not change with respect to the wavelength but the mu changes. So we could write the focal length of the convex lens as convex as 1 divided by mu of lambda - 1 times some k convex. Similarly the concave we can write it as 1 divided by mu of so this is concave, and this is convex, and this is concave mu of lambda -1 times k concave.

So using that using this in the above expression we can simplify this into $f_{\text{combination}} = k_{\text{CC}} \text{ times } k_{\text{CV}} \text{ divided by } \mu_{\text{CC}} - 1 \mu_{\text{CV}} - 1 \text{ times } 1 \text{ divided by } k_{\text{CV}} / \mu_{\text{CV}} - 1 + k_{\text{CC}} \text{ divided by } \mu_{\text{CC}} - 1$. Now when we do that, we can you can see that this whole expression will boil down to divided by this will get cancelled with the net denominator that comes out here leaving $k_{\text{CV}} \text{ times } \mu_{\text{of the convex concave lens}} - 1 + k_{\text{CC}} \text{ into the } \mu_{\text{of the other lens}} \text{ minus } 1$.

So now if you differentiate this with respect to lambda because so far and we set that to equal to 0 then you can actually see we need the functional description of this mu's right the refractive index.

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So, using dispersion relationship we would be able to write an expression given a material we would be able to write an expression for mu of a given material CC or CV as a function of lambda okay. So, as a function of lambda and its called one of the equation its called as Sell Meier's equation but it does not matter. So, the idea here is you need to be able to write this as a lambda and then for a given choice of materials of mu CC and mu CV.

Then what you can already see is that you would be able to set whole expression to 0 for a fixed value of the different refractive indexes, different radius of curvatures giving a net focal length right f this is not mean this you still have a net focal length given by f_{CC} / f_{CV} by this thing is this still can be positive or negative whichever we wanted. So, that you have a complete flexibility on this depending on how you choose your radius of curvature.

So by choosing appropriate radius of curvature along with the materials okay in a sense in short one can choose a high dispersion okay yeah high refractive index convex lens and a low refractive index concave lens I am sorry the other way round. So can actually get rid of this axial

shift that the chromatic aberration that we have talked about and when you do that since that f of this function f depends on the nature of this f of λ .

You may be able to I mean you have to choose for which point which 2 points actually you are trying to correct for because the R_1 and R_2 are fixed numbers right. So it becomes you can only approximate and you can kind of approximate and solve it exactly for 2 wavelengths and people do that and when they do it correct for 2 wavelengths and you call them the lens the resulting lens as Achromat and the correction we call it as a Achromatic correction okay.

So you choose mean in sense you are only correcting for 2 of them so what we do is we people do choose the end of the visible spectrum. So if they choose 1 blue wavelength and 1 red wavelength. So it is basically corrected around 460 nanometers and I think it is around 600 okay we do not have that number here. So, 600 and I think it is 15 nanometers I will say 630 nanometers okay.

So that so when you do that then you call that I mean that is to correct two wavelength corrections you call it as a Achromatic correction slightly better versions the of this lens is Apo Chromatic correction you need to introduce more elements okay with there is no magic here because we have 2 of them and then we played with that variables we know that if you solve for it then you can correct for 2.

But if you were to have you want to correct for our overall longer region 3 wavelengths you need to have more elements then they are called as Apochromat and one of the Apochromat they are corrected for 3 wavelengths for 460, 540 and 630 and there are also different kinds of a correction introduced by using material called as a fluorite as a glass and when so the they are so when we are actually looking at a wide spectrum right you are not like a fluorescence spectrum and stuff.

So Achromat and Apochromat be very good if you are actually looking for a specific wavelengths okay. Now if you are looking for a wide spectrum and their the transmission several other aspects are coming into play you might look into a fluorite based lenses and they are

basically different in their material properties compared to Achromat and Apochromat. So in the Achromat and Apochromat the lenses that we people in the Achromat specifically the 2 lenses that people use are one is what is commonly called as a BK7 or a crown glass.

And that is where you make your con this one was BK7 or a crown glass and the concave is made with Flint glass or also called as SF2 high dispersion. So some high refractive index and as a result so what the way you can think of is that the extent of the refraction that the bi convex lens produces right is compensated by this high refracting SF2 lengths the dispersion is compensated there by pushing the violet light forward meaning the focus forward and then bringing the red and violet in the same place.

But that if that is not sufficient then we have to use more multiple lens elements, and which result in Apochromat as we go from that from here to here oh actually not from here to here. As we go from Achromat to Apochromat the number of elements really goes up and that transmission goes down okay the transmission is high for Achromat but its lesser for Apochromat because of this multiple wavelength connection. Today the modern-day microscopes objectives they do correct for it but then this is a very strong limitation.

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The image shows a handwritten table of microscope objective specifications within a software window. The table lists three types of objectives: (i) Plan, (ii) Plan Apo, and (iii) Plan Fluor. For each, it provides magnification (4x, 63x, 25x) and numerical aperture (0.13, 1.0, 1.05). To the right, it lists 'Vis-IR' (Visible-Infrared), 'MP' (Microphotography), and 'Wavelength'. Further right, it notes '(Air objective)', 'W' (Working distance), and 'Type of immersion media'. Below the table, it lists tube lengths for Leica & Nikon (200 mm), Olympus (180 mm), and another Olympus (165 mm).

Objective Type	Magnification	NA	Vis-IR	MP	Wavelength	(Air objective)	W	Type of immersion media
(i) Plan	4x	0.13						
(ii) Plan Apo	63x	1.0						
(iii) Plan Fluor	25x	1.05						

Brand	Tube length
Leica & Nikon	200 mm
Olympus	180 mm
Olympus	165 mm

And as we as you can see in our objective list the table that we have made, we had 2 different kinds of objectives that 25x the long Olympus one was is a Fluor objective why the Zeiss one

63x that we had is an Apochromat all right. So, while the plan is not corrected for any of this chromatic aberrations okay. Now what this plan means, we will see it what this plan means and what are the other aberrations that can come rise or that can be present in an objective lens. We will see in the next lecture.