

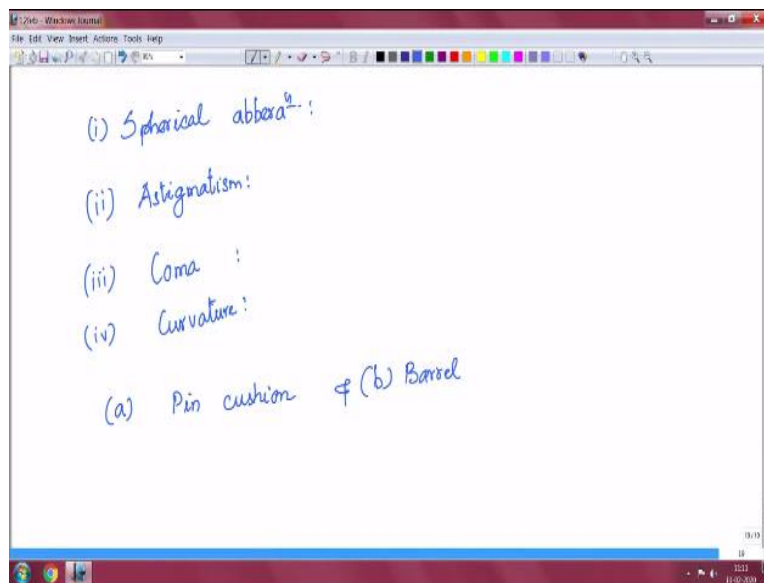
Optical Spectroscopy and Microscopy
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Lecture – 53
Fundamentals of Optical Measurement and Instrumentation

Hello and welcome to the course on optical spectroscopy and microscopy. In the previous lectures we were discussing extensively about the objective lenses and what are all the aberrations that can happen in these objective lenses and how do we understand these aberrations, aberrations and the corrections that are incorporated for these objective lenses. So we were talking about chromatic aberrations so far which is aberrations that are arising because of we trying to see through the objective multiple colors at the same time, okay.

Here the; in this lecture what we will focus on are the aberrations that are present irrespective of the color. We can also call them as Monochromatic aberrations because they are in; the extent of the aberration or the presence or absence of it does not depend on the color but is the nature of the lens itself is going to produce that. Now what are the kind of aberrations here that we are going to be focusing on?

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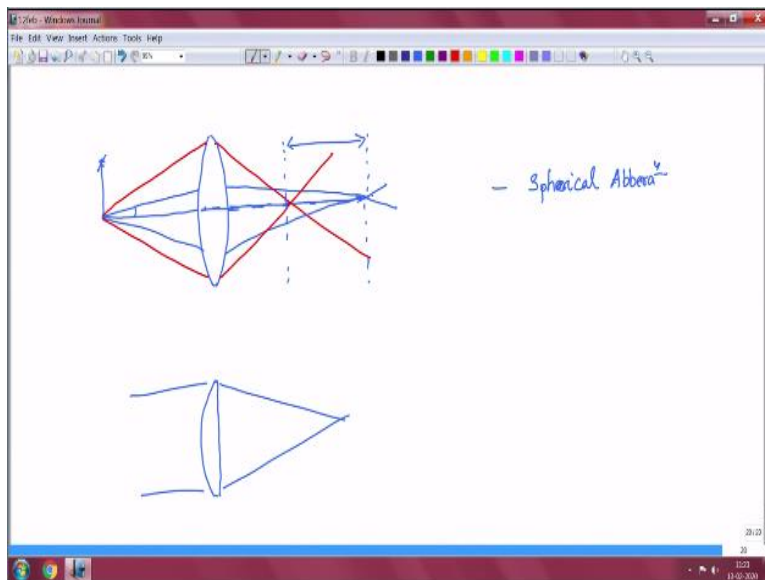


We will be talking about Spherical aberrations, Astigmatism, Coma and Curvature. Along with this we will also be talking about two other kinds of aberrations called as a Pin cushion and

Barrel aberrations. Now these by themselves are not necessarily a separate class they are though talked in various different lectures and the courses as; it is an different aspect though the fundamentally they are a combination of some of the other some of the other nearby some of the other fundamental aberrations.

You can express these or you can understand this in terms of that, so in that aspect optically they are not very; they are not a separate class. However, in the; during the manifestation in their images and stuff they come in way too often so it is good to talk about them and understand where they originate from, okay. So let us start with spherical aberrations.

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The one of the fundamental assumptions about we writing down the expression for the focal length is that the light incident on the different, different parts of the lens all behave exactly the same, there is not much of a difference. However, that you will see (()) (04:35) is a very strong contradiction to start with itself. The contradiction goes pretty much like this. If you had to assume a point source or then there and it is close by.

Then the angle that an incident ray substance towards the periphery is very different from and around the central optical axis. So since the incident angle is going to be different the extent of the refractions produced by this lens will be; are to be different. Now when we wrote down those

previous expressions there we make an important assumption that this angle, angle that we are talking about is very, very small, the angle subtended is very small.

So small and the objects that we are talking about as an infinitesimally small objects, we really do not worry about there is no extent that we are talking about here, so we are not talking about an object that is like this so where we have rays originating from different parts of the object meeting the lens at; in different angles. Even with in here we are going to; we will; we would have restricted ourselves to a very, very small angle it is; we call that as a Gaussian approximation because Gauss actually developed this whole optics geometric.

So we are looking at Gaussian we were looking at Gaussian optics. The moment we go away and I mean go to real system go away from the Gaussian optics you immediately realize that the extent of the refractions are very different, okay. So what is going to happen is that the light will take; the light that are; the light rays that are coming closer to the focus are; so the; that are closer to the central optical axis focus at a different place compared to that of the light rays originating from the periphery, okay.

So this gives rise to light rays coming so we will continue here. So the light rays coming; originating from the periphery, all right so we can throw that here, so they; what I'm going to do is I'm going to use different colors to represent the; color does not here mean the wavelength but the different colored lines they represent the spatial segregation, right. So they focus longer while this originating from the periphery focus closer to the lens.

So what you can see here is that the focus is spread over a larger space a series of planes in the Z along all along the axial dimension, resulting in an extended shape of the object with a characteristic feature we that we call it as spherical operation. Now as you can see this, this is because of the fact that the, the refraction of the peripheral rays are very different from that of the axial race. One way or the peripheral rays being more refracted than the axial rays.

And naturally the by convex lens is more prone to such kind of aberrations. One way to actually avoid that is to employ what we call as a combination of a biconvex and the Plano-concave in

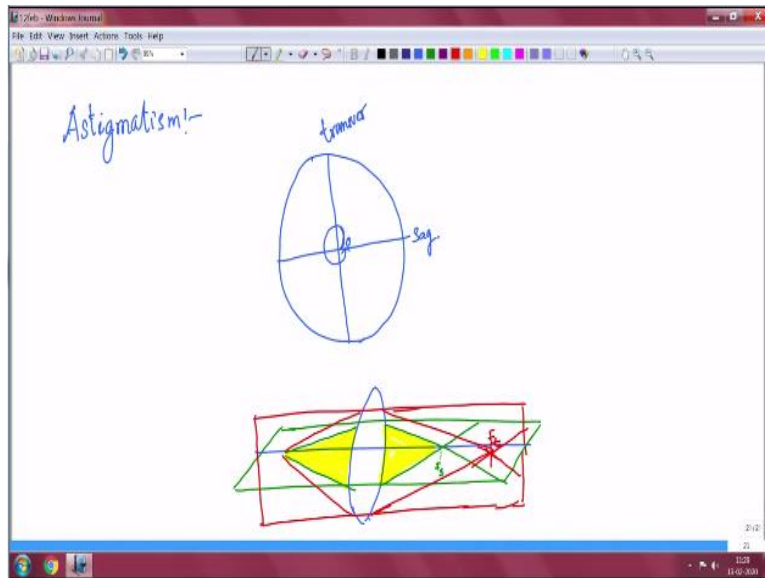
here it is really important to have a Plano-concave or a Plano-convex lens itself. This to a greater extent puts I mean takes care of these differences in the focus and such combinations right combination of using a biconvex lens and that Plano-concave or a Plano convex lens is one way of correcting the spherical aberration.

And of course we can always restrict the aperture but then that comes at the cost of not being able to efficiently focus the light and also be able to not being able to collect the light more efficiently. So in this system you would expect the all the light; I mean all the rays to be converging in a space much more closer in the along the propagation axis than that is present in the biconvex lens.

So what in an objective lens you will see is that wherever they had to use a positive meniscus they would compensate by using in one side Plano-concave lens or to accommodate for these spherical aberrations. And so the; again as you can see that the number of elements that starts to that keeps adding up as we start to correct for each and every one of these aberrations. Clearly, this is a vital aberration that we need to correct for to get the best possible focus, any; if the effect of such aberration is that our localization accuracy is now compromised.

Because the light photons can be anywhere between this point to this time this region so you would not be able to; so that the minimum focus is somewhere in between, so the optimal focus would appear as if it is somewhere in between but it is much larger than I mean in the lateral dimension than what you could have achieved without a spherical aberration.

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Now next in line of this aberration is our astigmatism. Now this is a result of when we actually look at the lens through. So this is as a result of differential radius of curvature the lens is processing in; or orientation dependent radius of curvature the lenses processing. So to define it let us take two axes so now do two mutually perpendicular axis, so let us call this as; let us just call this as transverse and let us call this sagittal axis.

So if the radius of curvature of the lens is not uniform or not symmetrical along this theta direction right when you go from; when you go from here to here along the theta you would expect that the radius of curvature should be uniform. Now for some reason if there is a difference in the radius of curvature due to manufacturing defects the radius of curvature along the transverse axis is different from that of the sagittal axis.

What you will expect is that the light that is originating that is getting focused by the; so let us write down I am going to flip the lens now, so okay. So I am going to draw two planes, okay. So a plain green along the; what I call it as a sagittal axis and pink, okay. So the green is the sagittal plane or; so we will; it is along this axis. Now if you take or maybe it is better not shaded, if you take light ray that is originating from here along this.

And if they were to come in focus at a point F_s in the sagittal plane while the light originating light rays originating from the and transverse plane so; of the same object, right where to come to

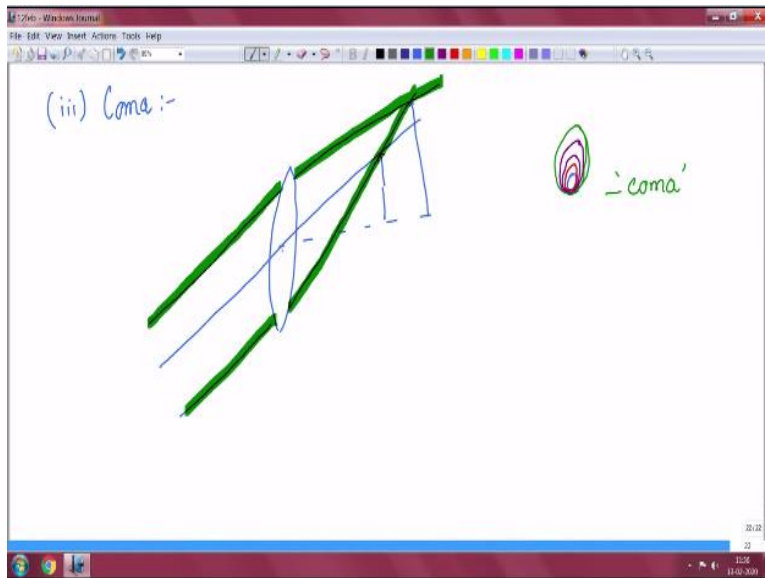
focus at a different point, okay. So now what we could do is that we could actually try to highlight these regions, so I will try to see if I can do it. So that is the sagittal plane focus. Now because it is a plane now the point; no it is no longer a point here.

But it is going to look like a line in the; sagittal plane is the horizontal plane in the; horizontal plane, it will be a small line in the horizontal plane, okay. So let us mark out this region, this is a transverse plane focus. So the; there will be a line that the point will become a line along the sagittal plane while the point will become a line along the transverse plane too but at a different distance different plane, okay, so sorry, okay.

So now as a result what, what we would see is that when we; the object is actually will look extended or distorted in one of its dimensions depending on which of the focal length is a larger you might look; it might look as if it is a stretch in its height or stretched in its lateral dimension because the focal length is different so the magnification is different and on top of it you will also see that the round I mean a circular point becoming a long elongated in addition to that, then the extent of elongation will also be different that gives, giving rise to the thin and mean thin and longer versus the fat and; fat and short images.

So now this had to be corrected by the really polishing the surfaces of the lens and taking I mean taking care of the uniformity of the lens by itself, all right. So essentially this, this has to do with the proper manufacturing of the lens itself so; and we sending the beam along the optical axis to a greater extent.

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Now the third kind of aberration that we could focus on is Coma. So this originates because of the peripheral rays in a lens coming to focus at a different distance; at a different distance in the focal plane than that of the central beam that is if you were to take a lens. Now let us take three rays. So we have an optical ray that going through his optical center. Now if the rays originating from the periphery were to converge at a different place along this axis, okay.

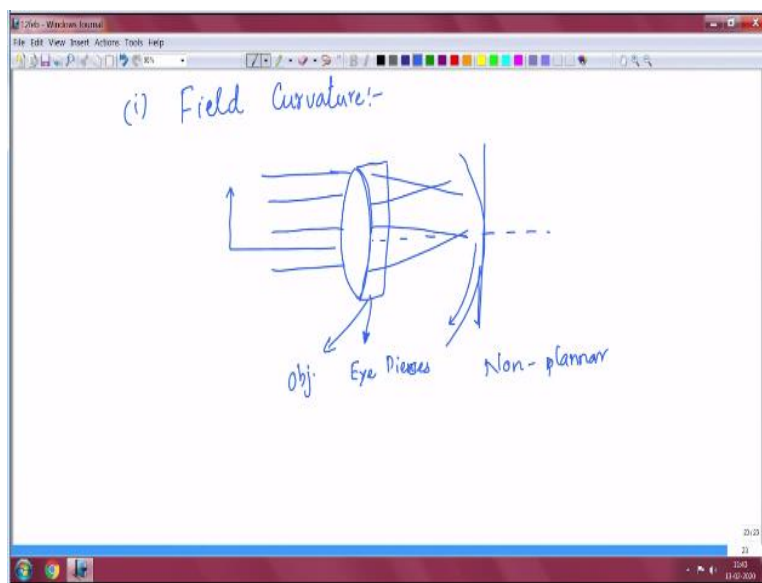
So let us take a ray that is coming here and it were if it were to converge at; there is a symmetrical axis on here. So now if it were to converge at this point while the; this is closer the central axis is closer to this while; now you can see that there is a difference in the; there is a lateral shift in the focal points of the rays originating from the periphery. So let me color it again with the different highlights.

These are the rays originating from the periphery, right compared to the blue. So what you see is that this green or the peripheral rays are focusing further than that of the rays traveling closer to the optical center. As a result, when you look at the image right about here you would see a strong pretty sharp and a crisp image that let us say of a dot will be a circle. But as we as we have as we go further; because the object have a finite size and then there are many rays around this.

So as we draw different rays you would see there each one of them would come and form an image above the optical center at slightly different with a slightly different centers ending up with the final green structure which is quite big because the focal length is larger. So it is; it will be very, also diffuse. So giving rise to this characteristic coma kind of an as a like a extension; circle becoming a very extended one sided at oval or a coma and we call that as aberration coma aberration.

Basically, there is a variation in magnification with respect to the aperture. So there are two ways to correct it, one is to not have these rays coming at an angle so they are passing; they do pass through the lenses at a; along the center, one. Number two is to reduce our aperture of the lens so that you do not have this ability to come at various different angles and there is no difference in the magnification because of the extended aperture because the peripheral rays have a different focal length compared to that on the central rays. Now these effects do take care of the aberrations that we have listed.

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Now apart from this we have is; two other quantities I talked about which is Pin cushion and a Barrel. Oh sorry, before this we need to talk about one more aberration, sorry. It is; this is due to it is called as a curvature, field curvature. So one of the important assumptions that we made is that when we are having an extended object which is not a point object then while; then that

image the points in the several points in this object would come to focus on a plane in a; forming at the focal plane forming a nice little extended object.

But if you actually trace out the ray diagram you will realize that the focal plane the planarity is not guaranteed particularly when you have very, very high radius of curvatures the lenses with high radius of curvature the plane instead of appearing straight I mean appearing as a; appearing perpendicular to the horizontal axis and then the optical axis and uniform. It starts to acquire an curvature the imaging plane itself is more like this which is essentially the different parts of this object comes to focus at; the arrows starts to up here, I mean if it is; if it were to be exactly so (()) (28:41), the arrow starts to appear with a curve, curve in it.

So this is, because of the fact that if you think of; a bunch of rays that are coming in from the periphery they would focus somewhere around here or in this and so on describing a curved surface, all right. So now that gives rise to the field being non-plannar, no field here is the, the focal plane being non-plannar. This is a very vital aberration which we need to take care of especially if you think about we having to scan the laser beam in a laser scanning system.

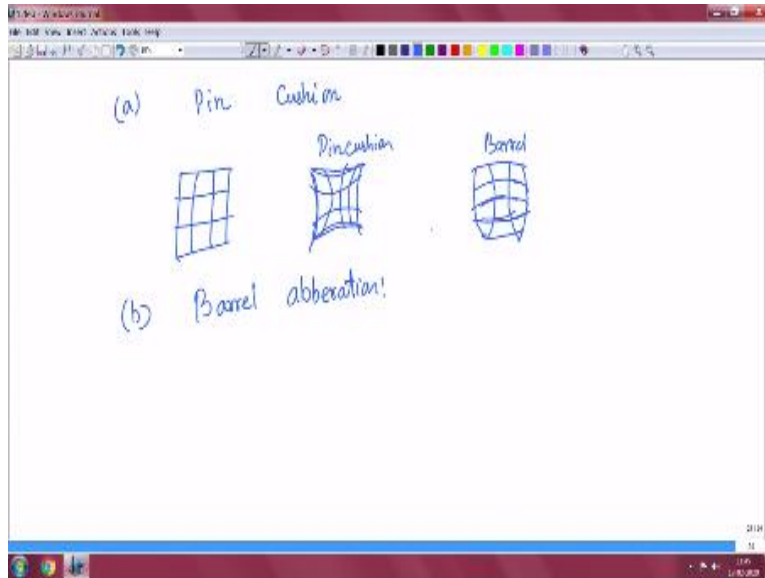
What we are actually doing is we are taking a parallel beam of light and scanning it across the lens surface at some point or the other. So on the important assumption there is that when it is getting scanned the focal points do travel as up travel in a plane not as in a circle or not form a surface of a sphere. So in such a case then what we will see is that the sample that we are imaging will not be a cube that we will image but instead it is a bent structure.

And then it gives likes to several different optical aberrations. So to avoid that, what we do is we use lenses that are corrected for this non-planarity. Again the correction is very simple, what do you do is that you use a combination of the; a combination of plannar; mean biconvex and the Plano-concave lens and that are pretty much ensures planarity. And you see these corrections these are very vital corrections.

These corrections are taking place at several points and you would see that in an eyepiece of a objective these are called plannar eyepieces and also in the objectives too. Now it is this

planarity correction that we see happening are mentioned in our objective list. If you go back in our objective list on the table that we are that we do here. So this plan here right we are talking about this plan here. These are represent that they; these objectives were corrected for forming the focus in a plain not in a curved surface, all right. So that is; that is about the field curvature and then how we correct for it.

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And related to the field curvature and the spherical aberration is; are these two other aberrations often talked about which is Pin cushion wherein if you place in the plain grid of squares in the image what you will see is that the central squares are correct but while as we go move away they become more curved, okay. So if you would see something similar to this. So while the b is; oh sorry this is barrel aberration, barrel aberration the picture that I have drawn is actually barrel aberration while the pin cushion is about as a grid that you have we have placed that is a square grid, planar grid would transform into one of this.

So this is called a pin cushion and; so if you see any of this then the; this happening in your microscope then the immediate thing that you need to look out for is either the field curvature is not maintained properly or it is usually it is a mixture of both the spherical aberration along with the field the flatness of the field not being maintained that gives rise to these aberrations. Now that concludes our extensive discussion about the objective lens.

And what we have done so far is in bits and pieces discussed different parts of the microscope and what are all the features that we; that they bring in and what we need to pay attention to. What we will do is that in the next class is that we would put together this all of these into an optical system for; and I will actually present to you a picture of how it looks in a very simple optical system when you put all of them together, how does an optical schematic look like.

And we will just briefly go through that before actually going and seeing in detail; how we can use such an microscope body to in an in a confocal microscope and how; and some of the image forming aspects of confocal microscope. We will discuss in the next lecture. Thank you. See you in the next class.