Optical Spectroscopy and Microscopy
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Lecture – 45
Fundamentals of Optical Measurements and Instrumentation

Hello and welcome to the lecture series on optical spectroscopy and microscopy, we have been looking at laser scanning systems and the imaging process itself, how image can be considered as representation of a contrast variations across the sample, we are actually capturing the contrast variation present in the sample across space and then that representation you call it as an image, alright.

So, now we said that if you want to capture the image with best possible resolution and now sensitivity, there are a few criteria that you need to meet; one of them is a how to go about sampling it, second aspect of that is to bring in how are we going to actually be able to achieve this in a real world in the instrumentation I mean, basically the optical instrumentation itself.

The simple and the trivial case first off is to say that okay I am going to use the lens and then focus it down and move the sample that I am going to introduce the relative motion between the lens that is focusing the light and the sample itself thereby, actually making the measurement of the contrast as a function of space alright, so that is the simplest possible system that you can think of whether problem with that is the speed.

And it is very limiting when you actually go ahead and calculate and see how, I mean how slow it would be, so as an alternate I said that you can actually scan the laser beam or move the light itself. Now, what is the, I mean and I said that it has its technical challenges in when we try to implement that so, let us first see what are the technical challenges?

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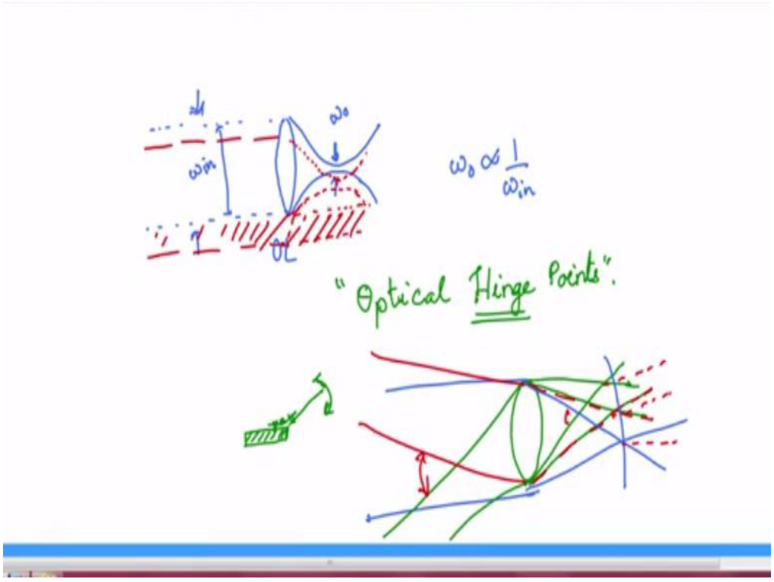

Number 1 is that we know for the optical system that we are looking at so, like an objective lens we are talking about here and we are drawing here and the optical system we are looking at will require will demand that we come in with a light whose incoming beam matches that of the aperture of the objective lens alright, the reason being that the wider the incoming beam right, the shorter or the narrower is our omega 0, the spot size, okay.

So, we want omega in, we know that omega 0 is inversely proportional to omega in, so larger it becomes the smaller and there is of course a limit I mean, so the limit being that it has to be; it can be as big as that of the objective lens, any bigger than that you are actually losing the light and you are not gaining anything, so you can have the width of the incoming beam as wide as that of the lens.

So, once you have that then you also want to be able to move it right, so the simplest case; simplest way you can think of moving would be to be able to translate the beam but you quickly realize that may not be a great idea here, right because you think of the dotted line represents the position of the beam at t equal to 0. Now, if you think of translating the beam when you do translate, the expectation is that the focal spot will also be translating which is good.

However, what you will be doing is that I am going to represent that with a dash line with the different colours okay, the width of the beam is exactly the same right, you are not changing the width and as a result, what you are doing is you are wasting or you are not this many number of the intensity proportional to this area, if we take a cross-section and then I can actually measure this area, the fraction; that area fraction will not be going through the lens.

As a result, what you are seeing is that even though you are let us say you are measuring the fluorescence as a contrast, so even though the fluorophore at position corresponding to this orange beam right, so somewhere around here okay, even though the number of fluorophores could be exactly the same and environment everything else could be exactly the same but the intensity of the light that is reaching which is the number of photons that is making it through is smaller by this fraction, right.

These guys do not go into the lens, as a result they do not go into the; they do not get focused, as a result the amount of light that is presented at this place is lesser than what it was with the blue, the position indicated by the blue lines. So, now this introduces a contrast in an artificial manner, this contrast; what do I mean by that? This contrast does not exist in the sample but because of your movement and then the lens chopping of a portion of the beam as it moves, you are actually creating a contrast which is not there which is not naturally present in the sample.

So, clearly that will not be a true representation of the sample, so then what do we do; so the any movement that we can think of should be such that they do not introduce any of this artificial contrast, so it is clearly there is a way to do it, idea here relies on creating what are called as; what we call as optical hinge points, okay. We know that what a mechanicals hinges right.

The mechanical hinge is a device that connects the immobile part such as corner of a wall or end of a wall to a moving path; a moving path being here a door okay, now these hinges; these are called hinges because one end of; one side of it does not know, the other side moves but it is attached to the shutter, those shutter, so that it can actually move up and down, you can think of this kind of a motion going rocking back and forth.

So, now what it does is that; what the hinge does is that it enables the door to move in space without having to; without we having to resize it, otherwise imagine if the hinge were not to be there, we have to be able to move that and that is not possible, it is going to; we need to be able to; you will find it difficult to do that. So, similarly if you have actually were to think of an optical hinge point and the hinge point being on the lens.

That is about the lens the beam is going to move alright, so now if what does it mean, so now you can think of a lens okay, so now I am going to ignore the Gaussian optics, so I am going to use just a ray optics, just like our good old high school, 10th standard, so wherein we represent through lines, the as a radiogram. So, what we like to do is this; a light that is coming in from the lens, I think we should represent it by different colours.

So that is a imaging; image I mean, that is a sample plane and as you can see what is happening is that the beam starting from green to blue is moving on the; moving about the lens and at the same time, what you can actually verify for yourself is that the width is exactly the same, whether it is blue line or green or orange. So, what we have done is effectively have created an optical hinge around the or about the lens.

As a result, what you have is that all these beams are actually coming to focus at the same plane but at different positions, so this way you can actually create a sample space which has been sampled at different locations without having to move the excitation beam across the lens, so at the lens the beam is actually tilting right, it is just going from one end to the other and then coming back.

So, this behaviour of creating an optical hinge point, lets us image the sample alright, now how do we; what does the optical hinge point mean in terms of geometric optics or in terms of focus of the; focal length of the lens and etc., we will see that in a minute, so that will; once we see that that you will see that enables you to be able to create or make build an equipment that can actually utilize this ideas to make measurements of the sample in x, y and z, okay.

So, now let us take a look at this once more the lens and the idea here is we are going to understand how do we, I mean go about thinking about the hinge points, alright.

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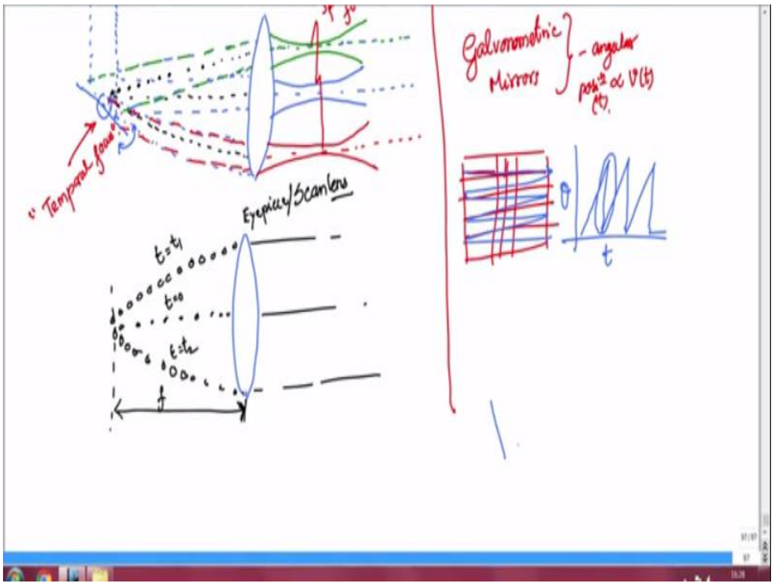

So, we will take a lens now, for this purpose let us look at beams that are thinner, it is exactly the same you can actually generalize but it is easier for you to visualize, so I am going to take a thinner beam. So, what we are seeing is that we are going to actually have a mirror reflect a beam of light, okay. Now, if you have a mirror that is doing this, we can think of or imagine in a thought experiment, what is going to happen to this position as we actually move this mirror about this axis, okay this rotational axis.

Now, you can see at some instant it will be high like this, at some other instant will be straight and at some other instant where the beam takes a path something like this, so I am going to just, I mean sorry for (()) (15:55) a different colour, so let us think of using; so now we can see these are the 3 different instances, so these 3 different instances the beam is hitting the lens at 3 different points okay.

So, now if I have not told you that this is a representation that is taken that is there because I am actually looking at the movement of the beam as a function of time, you could also think of this as arising from drawing light rays originating from this mirror and I am tracing multiple of them, okay. So, in such a case then we can ask a beam has a finite extent, so let us say I am going to draw the central axis of the beam okay.

The way you draw the central axis is let us take a black colour and you can write; you can think of the central axis alright, so now let us go back, if I just draw the black lines alone so you will see, so these are the 3 different time points, so let us call this as t equal to 0, t equal to t1 and t equal to t2, you can also go from 0, 1, 2 it does not matter. Now, if this mirror were to be at a distance f from this eye piece.

So, then let us know, I mean are it is also called as a scan lens, then this is; this amounts to light rays originating from the light source that is kept at a distance f, the focal length of this lens. So, the immediate property; immediate manifestation of that is to say that hey, this is originating from the focus, so then you would say that okay, they are at originating from focus which means, on the other side, they would be going parallel to each other, right.

Because any light that is originating from the focus and it will have its image only at infinity which means other words, they are the light rays are parallel to each other, so now that same argument holds good here too, so these 3 different points even I mean, these 3 different lines even though represent the beams at 3 different positions, you can actually consider that as a long time average right, long exposure snapshot of this whole situation.

So, when you do that what you are going to see is that the light rays are originating from the focus which means, it is going to come at a parallel to each other, so taking that you can think of when we actually move the mirror, this blue light is going to go from here and it is going to come out, so the central line corresponding to the blue going to be parallel to; parallel to what; parallel to sorry, this is the green line that is on the top, then you have a blue line.

And then orange line however, these beams also have a spatial, they have a spatial extent, so or in other words, they are the wavelets inside the beam they are actually have a definite phase relationship between them, so that is the point when you actually them through the lens how it is going to look like is; there will also be focused, right each of this beam will be focused.

But the point is the central axis of the beam all will be travelling parallel to each other, they would not meet unlike here, so here the original light was initially travelling parallel and then it met an optical setup, wherein you can actually say this is coming in as if that the light rays are originating from the focus, as a result the on the right hand side, you will see that the several beams coming corresponding to different times.

And each beam will we in focus or not depending on where you are actually plugging the (()) (23:32), alright. So, now this way of creating or moving from a representation where the beam is moving in space right, so I would appearing as if it is starting from one place, so we would, we tend to call in the lab, these points as temporal focus points and the focus related to these 2 or 3 is called as spatial focus.

So, now you could go between the temporal focus and the spatial focus actually, you will see that at this point the beams are all focused in space but they are all traveling parallel to each other. So, basically if you look at the movement of the beam it will be travelling equal amount of space, it will be covering equal amount of space in equal time at each of these times alright.

So, they are going linearly, so this on the other hand at this point, the movement is angular meaning, depending on how far you are actually looking at this or looking to capture this laser light, the difference between the beam positions would be different, I mean at close, the extent of the beam would be a smaller, while at the end the extent corresponding to both the functions will be larger.

So, now what you can actually see now is that suppose, if you were to have a mirror; a moving mirror right, these mirrors are called as; we will see galvanometric mirrors, okay so they will change their angular position is directly proportional to the voltage that is given, so you can think of if you want to change the angular position, you can change that their voltage as a function of time that will eventually result in an angular position being changed as a function of time.

So, now what we want to do is; we want to be able to move this beam back and forth in a rocking motion, so if you were to actually plot the position of the mirror; angular position of the mirror, you would expect it to ask the okay; I am going to recreate the grid space remember, it is a; it is called raster scanning, so in the raster scanning what you are actually doing is that you are taking in a beam and then moving it across, sweeping it across the x and; you sweeping it across the one axis, drops down in that manner right.

So, which means if you actually look at the angle subtended by this mirror, now that angle is going to keep increasing, falls back down when it comes back to this and increasing falls back down okay, it should be the drop is pretty straight down, so these are; so this is a function of time and this is a theta, so at these positions let us actually retracing the mirror back.

And you can see that you are changing it in a linear manner which means, the beam is going to travel I mean, equal amounts of sample, equal amounts of time in this space from one end to the other now, with that geometry let us combine with that idea, let us combine that and putting it in front of a lens. So, when you do that we will be able to create what are called as an optical hinge. So, we will see that in the next class how the by playing the; this galvanometer along with that of the focusing properties of the lens particularly, the scan lens, how we can actually create an optical hinge point at the back of the objective lens, alright, thank you.