Advanced Measurement Techniques in Fluid Mechanics and Heat Transfer

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Week – 08
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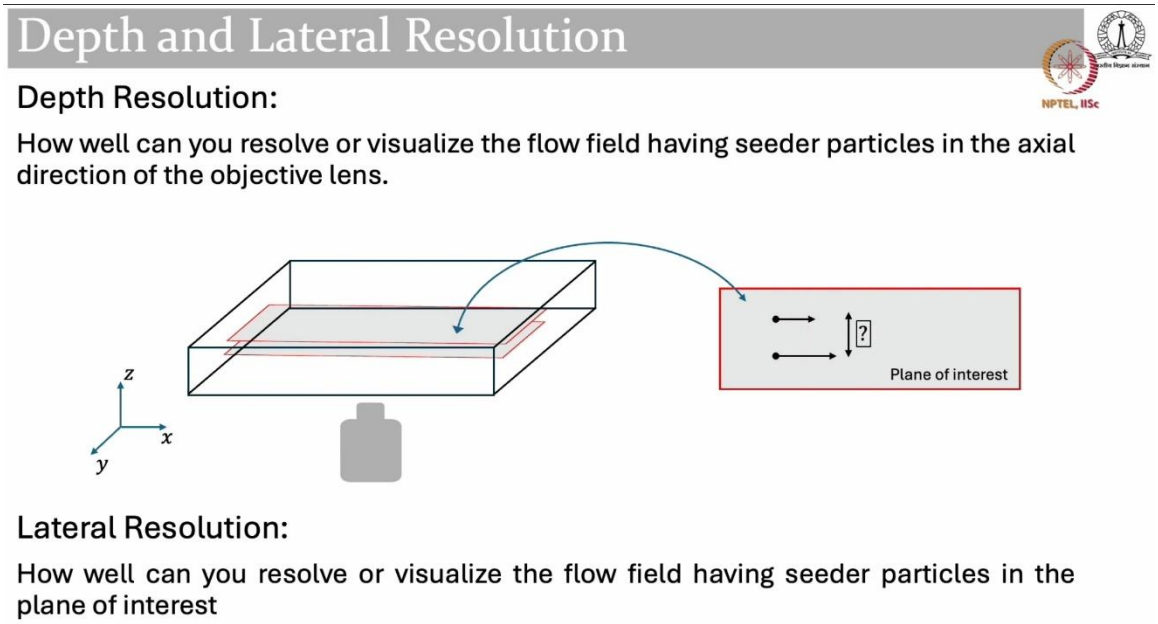
Micro PIV – 4

So, now we will discuss in details about the depth and the lateral resolution which we can obtain while doing micro PIV. So, since it is very different from macro PIV and there are other complications related to micro PIV systems regarding the resolution, we will discuss all those details in the upcoming slides. So, what do I mean by depth resolution? So by depth resolution, what I want to say is how well you can resolve or visualize the flow field having cedar particles, obviously in the direction of the axis of the objective lens; that is the depth of your channel. Now, if I show you a schematic here. You can see, suppose you have a microchannel like this with this coordinate system: the x direction, the y direction, and the z direction. Z is the depth, basically, and if you visualize the microchannel from the bottom, you see that you want to know how much you can actually see the particles in this z direction, like what the region is.

Till where or what is the plane in which you can actually focus all the particles very well that would give you the resolution in the z direction or the depth resolution, and the other thing is the lateral resolution. What I mean by lateral resolution is how well you can resolve or visualize the flow field having serial particles in the plane of interest. Now, for example, you have this. Now, if this is the plane of interest, then how well can you resolve the data of the particles using your optical system in this plane? So basically, like, yeah, you can see two vectors popping up here and how much distance you can get between those two vectors, or how well you can resolve the vectors, and this is very important because.

This is the thing you want to get accurate output from your data when you image the basic flow field using the camera and the cedar particle system. So, the output actually as I showed you before also that how well you can get the spacing between the vectors which to get meaningful results. So, again, it is very important to understand the capacity and limitations we face while performing micro-PIV experiments in terms of resolution. So, before starting any of the experiments, it is very crucial that one perform some mathematical calculations to understand his or her system: basically, what kind of lens you have, what the objective is, what the microscopic objective you are using is, what the magnification is, what the numerical aperture is, and what the depth of your micro channel is, as well as the material of your micro channel; all these things play a significant role in resolving your flow field, so we will be discussing those things here. So, basically, again,

as I told you before, how well can you resolve the distance between two vectors? That is what the question is.



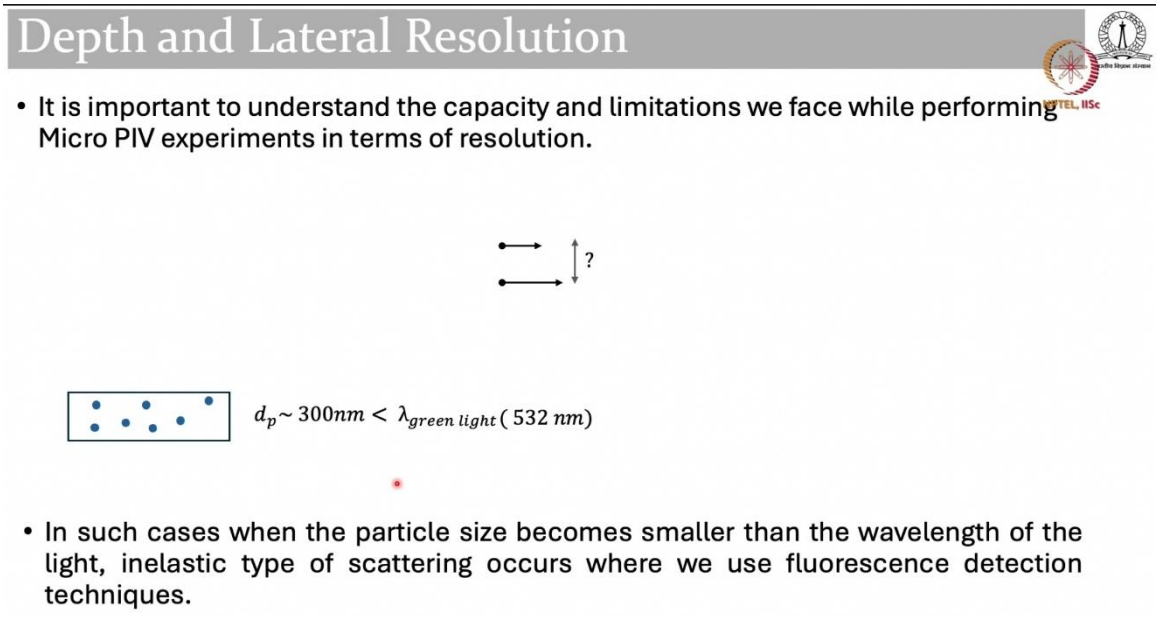
Now, suppose this is one of the interrogation windows and you can see some particles inside it. Now, the particle size in the case of micro PIV can be really small, as we discussed previously, that it can be in nanometers, and it can even be less than the wavelength of the light you are using to visualize them. So, suppose we have, if we use the green light to visualize that, do not go by the color of these particles because it would not look like this; this is just for visual representation. So, suppose the particle is around 300 nanometers in diameter, which is less than the wavelength of green light, which is around 532 nanometers. Now, normal scattering does not work here.

So, in such cases when the particle size becomes smaller than the wavelength of the light, inelastic types of scattering occur. That is where we use fluorescence detection techniques, and there are other techniques, but we would not go into the depth of others. And about inelastic scattering, I mean you can read about it, and basically, the internal state of the particle alters after it collides, and its normal scattering doesn't work. So this is one of the complications that we deal with by using fluorescence detection techniques. So, in comparison with the macro PIV system where the glass spheres we normally use or the tracer particles used are mostly on the order of 10 microns or greater, possibly 40 to 50 microns, which is much larger than the wavelength of the light, in that case, basically light means From light, when I say light, we normally by default assume it to be the green light, which has a wavelength of 532 nanometers, and in this case, we see Mie scattering, which is actually proportional to the square of the particle diameter.

So, if you increase the size of the particle diameter in the case of macro PIV, the tracer particles will also increase. So, a lot more scattering will take place. So, more light will fall on your camera sensor. However, there is again this catch where you cannot increase the particle diameter to a certain limit because the Stokes number would again become very large, and the particles won't follow the path faithfully. So, again, there is a trade-off, which I think Professor Basu has discussed in depth in his PIV lectures.

But yeah, I have brought it up here just to highlight the difference between the micro and macro PIV systems. So basically, if you have a test section like this, and now you see this is a large-scale test section, you can assume it to be in the orders of centimeters. So suppose this is like 10 centimeters by 10 centimeters, and then if you want to see a plane, you use a laser sheet, basically a green light laser sheet. To illuminate a single plane, you can see these particles very well in this plane, and basically, the light gets scattered perpendicularly to the direction of the camera, falling on the camera sensor, allowing you to actually record the image exactly in this plane. The thickness of this light sheet can vary from 1 to 2 mm.

Now this is all good for macro systems, but what about micro systems? In the case of micro systems, we don't even have the thickness of the channel as big as 1 to 2 mm, which is almost around 2000 microns. So what we deal with is like 100 microns, 50



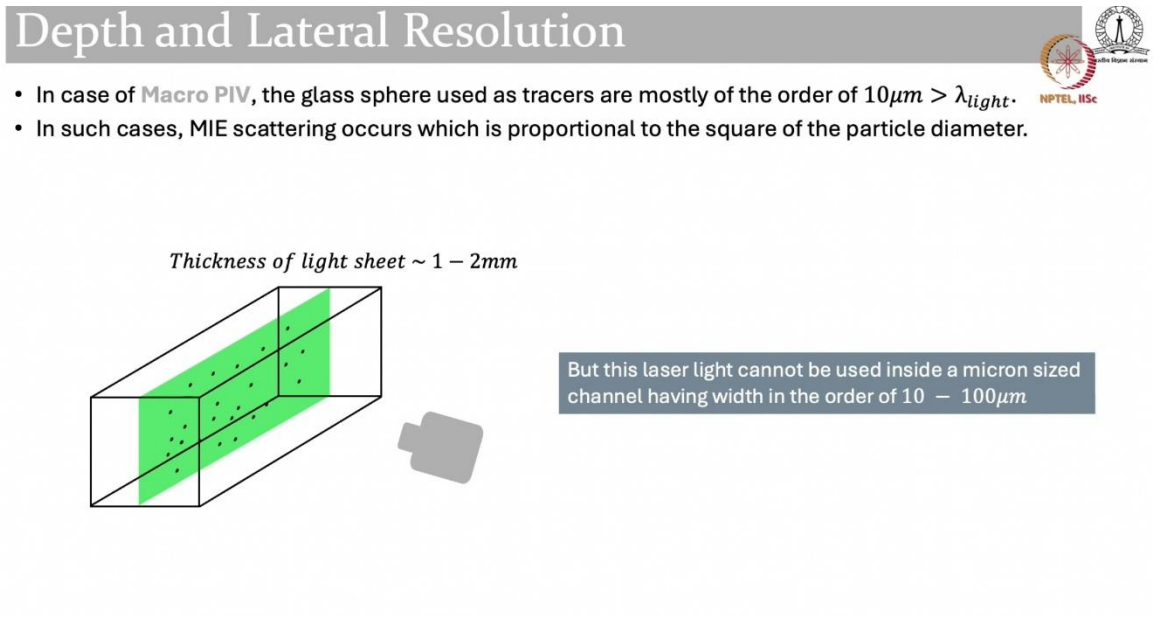
microns, or maybe even 10 microns many times. So, how do you solve this problem? If you use this laser light for your microchannels, basically on the order of 10 to 100 microns, the light will flood the whole channel, and you will see everything inside it. Right, so what you will see is basically a huge region that is not desirable most of the time. Instead of using a sheet, if you are able to modify the laser sheet to a thickness of, say, 10 microns or

even 50 microns, then aligning that small sheet of laser light with your microchannel can be very, very difficult because you are dealing with precision at the micron level.

So what is done instead is... A volume illumination technique is used. Here, a volume of the microchannel is illuminated depending on the needs of the experiment.

Like whatever depth you require, you can actually adjust it using some kind of optical modification. So, what I am saying, what I am trying to say is suppose you have a microchannel here and suppose the flow is a plane positive flow inside that, and you are looking, you have basically a laser system which is behind this, basically that is the bottom, and you are looking from the top, so you can say that at any section, say suppose at this section, say. Suppose, in this section, you have a profile like this; it is the classical parabolic profile where you have a larger velocity at the center, and due to the no-slip condition, the velocity diminishes near the walls. Now, if you see this whole region, what you will be seeing, what you will be capturing, is particles. Of varying speeds across the depth.

I mean you will also capture particles moving much faster and particles moving much slower. So, what you will actually get is a depth-averaged velocity field and not an in-plane measurement, but many times we need the velocity field in a single plane. So, we need to concentrate on a smaller area. Now for this, what we can do is modify systems



like we modify our optical system, which allows us to focus only on a narrow plane. To illuminate a small region of the microchannel, we need to find out the depth of field of our

microscopic objective.

So, we will talk a bit about the concept of depth of field. So, suppose you have a point object here, I will just use my pointer. So, suppose you have a point object that is here at the object plane, and the light that is coming through this is basically the lens. The lens, I hope, is clear, and this is the sensor plane or sensor of the camera, or the image plane where you want the image to be formed. So, the light rays will go through this lens, and it will form an image at this point.

Now, let us assume that this point, this object plane, is exactly at that point. where it is actually forming a very sharp and focused image of the particle. So, this should be the arrangement for it. Now, suppose your particle is shifted a bit away from the lens, right? So, now if you draw the light rays, it should look like this. What will happen is that the rays will start to converge a bit earlier than the image plane, and you will get an image that is a blurred image on this sensor or the image plane, which would look something like this, and we call it out of focus.

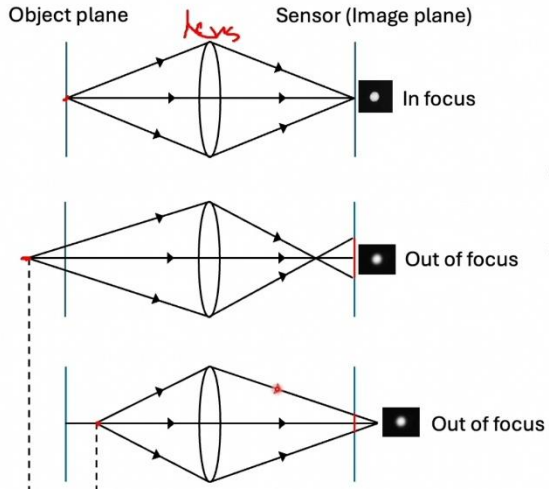
Another situation that can occur is if your particle is brought closer to the lens head and not at the object plane; then, if you draw the rays, it will look something like this, and the image and the rays will actually converge somewhere behind the sensor plane or the image plane, resulting in an image that is again blurred and out of focus. So, now the way we define the depth of field is that the distance between this displacement of the particle from the object plane and this displacement near the lens is given as delta, and this is basically the depth of field of the objective. But how do we actually define it? I mean, how do we know, yes, that this delta is enough? Because you can take this particle much farther back, and you can take this much nearer to the lens. So now this is a trade-off again, and you have to decide that, okay, suppose until this distance, if your particle is somewhat in focus and which is basically acceptable, then you can use that as one of your limits. And even in this case, when you bring the particle near to the lens and if you are getting an out of focus, but it is.

.. It is somewhat acceptable in the acceptable range; then you can consider this to be the other limit. So, for a range of length delta, these images are in the acceptable range of focus, and from here to here is basically the distance known as the depth of field. So, basically, we will assume that the objective will be able to focus over a depth of delta. So, again, now we have found out, say, suppose our delta is some x microns. So again, the depth of our channel has to be greater than x microns so that we are only able to see a small volume of

the microchannel, or a small focus plane of the microchannel, and not a very large section. So for that, delta has to be smaller than the depth of the microchannel. So now we'll see again, suppose a bit more about the depth of field. Suppose you have this microchannel of height h, and this is the coordinate system. If you are viewing it from the bottom, what I want to say is that this is the depth of field I am talking about. I mean, you can see a bit of a gap here, and this is the region.

Depth and Lateral Resolution

Concept of Depth of Field

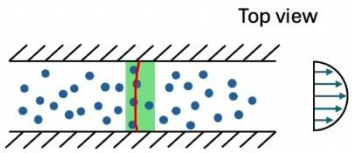


- For a range of length say δ, the images are in the acceptable range of focus
- So, we need to choose an optical system such that the value of δ is smaller than the depth for the micro channel

For which you are able to basically focus the particles, and in a two-dimensional figure, you can see something like this; this is basically the depth and delta, and now you can see that you are able to view only this much of the region and not a much larger region. So,

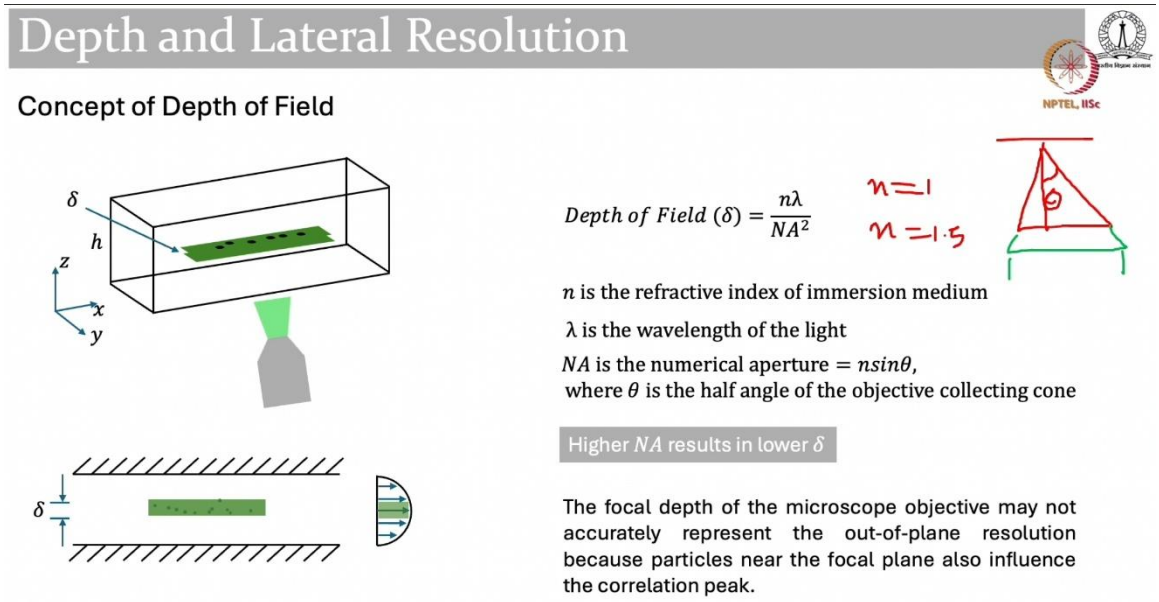
Depth and Lateral Resolution

- We use volume illumination technique.
- A volume of the microchannel is illuminated depending on the need of the experiment.



- So, what we can measure is a depth averaged velocity field.
- However, if we need velocity field in a single plane, we need to concentrate on a smaller region.
- For this, we modify the optical system will allows us to focus only on a narrow plane.

this will give you much better results over a smaller range of volume. And if we start to define the depth of field mathematically, then the depth of field is actually given as n times



λ divided by n a squared. What are these parameters? So, n is the refractive index of the immersion medium. So, normally immersion medium is used on top of this objective; this area where I am highlighting actually helps to increase the numerical aperture, and this n is equal to 1 if there is nothing but air.

And this can actually be around, say, 1.4 to 1.5 if you are using some oil immersion on top of this objective lens. So this is very important, and you can change these things as per your need during your experiments. λ is the wavelength of the light, and normally we use green light, so it's around 532 nanometers.

Then the numerical aperture na is defined as n times sin theta, where theta is the half angle of the objective collecting cone. So, basically, if you do something like this, no, I am sorry. or something like that. So, this angle, basically—pardon my drawing a bit—this is the theta, this is the half angle, and basically you will have your objective here, somewhere. So, this angle is defined as theta, and the numerical aperture is actually the product of the refractive index of the medium here, the medium in between here, and the sine of this half angle.

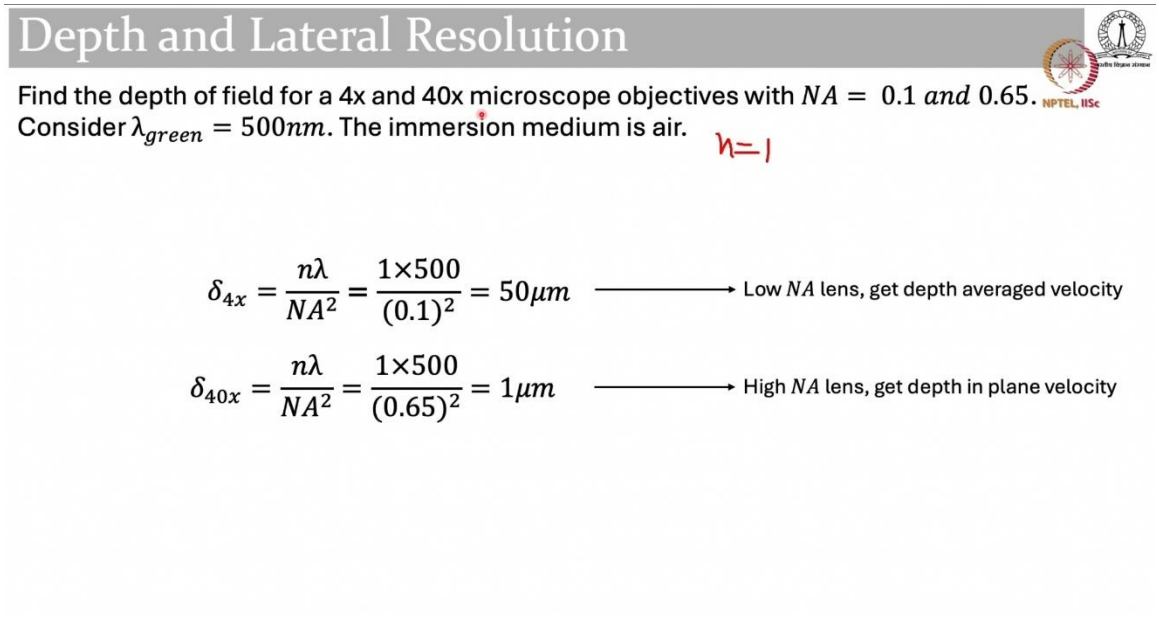
So, from this relation, you can see that if you have a higher numerical aperture, the depth of field is lower, and if the depth of field is lower, basically you can see much smaller features inside your microchannel. The numerical aperture is nothing but the light-gathering power of the objective, and normally we prefer it to be larger, but again it has its own pros and cons. So, now this is really important that is why we are studying all this because the focal depth of the microscope objective may not accurately represent the out

of plane resolution particles. And that is because particles that are near the focal plane— I mean, say you are focusing somewhere here. If there are particles that are slightly up near this focal plane, they can also influence the correlation peak; thus, you can actually get some illumination from those particles, which can increase some kind of noise or something like that in your correlation peak, so you have to take care of this while designing your algorithm.

We will talk a bit more in depth in the upcoming slides. So, now let us find the depth of field for some of the microscopic objectives that are commonly used. We will find a 4x and a 40x microscope objective. With the numerical aperture values of 0.

1 and 0.65, respectively. We will consider a green light source with a wavelength of around 500 nanometers. The immersion medium is air, which means your small n, that is, the refractive index, is 1. So, to find the depth of field for a 4x objective, we use this formula which was discussed previously, and if you put the values, that will be 1 into 500 by 0.1 to the power of volts, you will get a value of 50 microns. Now you can see that for a 4x objective you get a depth of field of around 50 microns.

So, it looks like a very large depth of field, but that again depends on whether your gradients, or say if the flow you are trying to visualize, are well within the depth of field. If the flow you want to visualize is, say, a larger, much larger size, around over 200 microns, then this 50-micron should be sufficient for you. However, in microfluidics, we normally work with very small depths of field. So, normally 50 microns give you a depth-averaged velocity field and not an in-plane velocity field. Again, everything is very subjective to what you actually want to see, and for a 40x objective, if you calculate, you will get around 1 micron depth of field, which is much smaller.

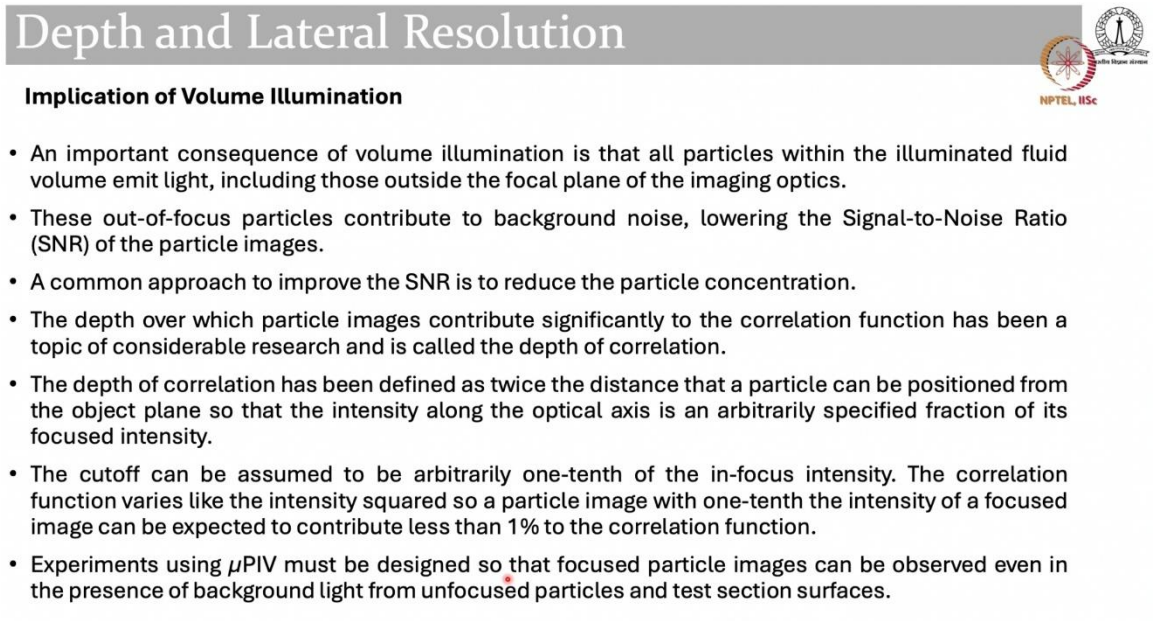


Here, you can actually focus on a very small region, and you can see an in-plane velocity field. So, if you have a low NA lens, you get depth-averaged velocity fields, and if you have a higher NA lens, you can see you get depth. In plane velocity, it basically depends on what kind of system you want to use for your experimental setup, and according to that, you can change the numerical aperture, change the objective, and even the green light source. Normally, we change the microscope objective and the numerical aperture by changing the effective index and those kinds of things. Now I will talk a bit about the implications of volumetric illumination.

So, by now you must have understood what we mean by volume illumination, and it can have severe implications because, unlike macro-scale phenomena, where you can easily get the data in a single small plane of interest. In the case of micro PIV, you are using a microscope objective that has its own depth of field depending on the optical system. So, it is very subjective, and it can be a tedious task many times to get the desired depth of field you want. So, understanding volume illumination phenomena is really important, and I will just give a brief talk on that. This slide will be a bit busy, but you have to read it if you need to know a bit more about these things.

So, what I mean to say is that an important consequence of volume illumination is that all particles within the illuminated fluid volume emit light. Including those outside the focal plane of the imaging optics. So, that is what we discussed previously: because of this volume illumination, the particles that are actually a bit out of the focal plane also start to emit some kind of light. And these out-of-focus particles can actually contribute

significantly to the background noise. And this term called signal-to-noise ratio is one of the most important parameters when assessing your post-processing data.

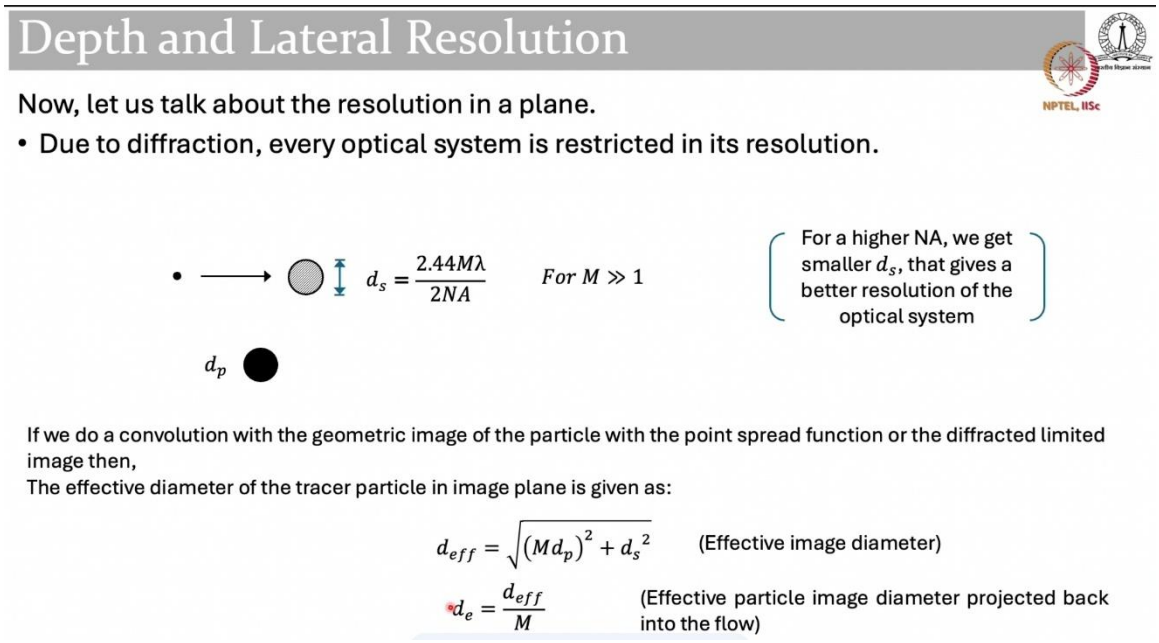


So, if your signal-to-noise ratio is very high, then it is better because that shows that the noise compared to your relevant signals is very low. If your background noise starts to pop up, I mean starts to become significant, then this SNR ratio becomes very small, and this can actually give inaccurate results. So one has to be very careful while processing one's data and understand this concept of SNR. A commonly used approach to improve the SNR is to reduce the particle concentration; or, if possible, you can also reduce the intensity of the light a bit, but that doesn't help much. Another option is to reduce the depth of your test section, but that is not always possible because you are performing a certain kind of experiment that requires a specific depth.

of your channel. So, normally the best way is to reduce the particle concentration a bit and then see if your SNR ratio comes in an acceptable range. Another concept that is really important, which I will be briefly discussing here, is the depth over which particle images contribute significantly to the correlation function we are talking about. And this depth is known as the depth of correlation, which has been a topic of considerable research. You can read many papers on this topic. Here we are talking about the particles that actually significantly contribute to finding the correlation peak.

So basically, this is the most important thing because this is what gives you the output that you want. Now, how do you define this? There are a lot of definitions you can find in the literature, but one of them is basically that the depth of correlation has been defined as

twice the distance that a particle can be positioned from the object plane. so that the intensity along the optical axis is an arbitrarily specified fraction of its focused intensity. So basically, there is a focused particle that has some kind of intensity; maybe let us call it the peak intensity. Now you define a fraction of that peak intensity, say one-tenth of that intensity, and until a distance where you get that much of the intensity, you assume that the particle should basically contribute to the correlation.



And if it goes out of that threshold, you basically discard it. So, let us say that the cutoff can be assumed to be arbitrarily one tenth of the in-focus intensity. So, say your intensity is 100 for something; one-tenth of that, basically, you can assume that below one-tenth of 100, things will be neglected. The correlation function, if you go into depth and understand how it works, actually varies like the intensity squared.

So, a particle. With one-tenth the intensity of the focused image, it can be expected to contribute less than one percent to the correlation function, right? Because there is an intensity squared relation, and since you are thresholding it to one-tenth, it will contribute somewhere less than one percent to the correlation function, and this much is something we don't want. So we can actually safely neglect it, and this will definitely reduce the Noise that was coming in the part was like the noise in the image, and it will contribute to and enhance the signal-to-noise ratio. So this depth of correlation is a very important topic, and one should also understand it; you can find a lot of information about it in the existing literature, which I am not discussing here in depth in the interest of time. So basically, experiments for micro-PIV must be designed so that the focused particle images can be observed even in the presence of background light from unfocused particles and test section

Depth and Lateral Resolution

Find d_e for a particle of diameter $d_p = 300nm$ using a 40x objective and $NA = 0.65$. Consider green light.

$$d_s = \frac{2.44M\lambda}{2NA} = \frac{2.44\times40\times532}{2\times0.65} \sim 40\mu m$$

The geometric image $= Md_p = 40\times300 = 12\mu m$

The effective diameter, $d_{eff} = \sqrt{(Md_p)^2 + {d_s}^2} = \sqrt{144 + 1600} = 41.76\mu m$

$$d_e = \frac{d_{eff}}{M} = \frac{41.76}{40} \sim 1\mu m$$

So, for this optical system, a $300nm$ particle will look like $1\mu m$

surfaces. So I hope you understand the meaning of this because now, as we have seen, there are particles that can be present in the background of the focused plane, and they can be some kind of reflections and other things from the test sections as well.

However, your experimental setup should be robust enough that the focused particle images can be observed even in the presence of these unwanted sources of light. Now we will talk about the lateral resolution and that is the in-plane resolution. We have discussed a lot about the depth resolution, where we saw the volume illumination technique and how you can achieve a desirable resolution according to your experiment by changing the numerical aperture, the objective, the magnification, and other optical parameters. So, here we will start talking about the resolution in a single desirable plane, so if you have studied optics previously, you must know that due to the diffraction of light, every optical system is restricted in its own resolution. By this, I mean that suppose you have a point object in the object plane and you want to see it on the camera sensor or the image plane.

You won't be seeing exactly what it is like. So, suppose you have some diameter; suppose I will just point with my pointer. So, there is a diameter at this point, but actually, you see something else that is basically another enlarged image of this, which is like a spot size feature, and this is actually the point spread function. For an optical system with a magnification greater than 1, you can define the diameter of this spot size function as approximately 2.44 times m, where m is the magnification of the system objective, and λ is the The wavelength of the light divided by 2 times the numerical aperture. So, what I mean to say is that you have a point-size image, but you won't be able to see it exactly; instead, you will see something like this, which is much larger than that point-size image.

We will do some illustrations to see how much larger an image we see in reality, and in

reality, say we have a point size; this basically means our particle size will be say d_p, so it will be much larger than this single point source. From this formulation, you can see that if you have a higher numerical aperture, you will get a small value of. This diameter and that will definitely give you a better resolution in the single plane. Because now suppose you have this, suppose we have this d_p, and if you are seeing this $d_p$in our optical system, we will see something larger than d_p, but we want to minimize that ds for that d_p, right, to get better or more accurate data.

So you can change the numerical aperture to reduce this ds. So now. As we saw, we can change the numerical aperture to change the ds to obtain better optical resolution of the system. Now, if we perform a convolution of the geometric image of the particle with the point spread function ds or the defected limited image, which is the spot size function, we can obtain the effective diameter of the tracer particle in the image plane, which is actually given by this formula. Say we call it "$d_{effective}$," which is equal to nothing, but the square root of magnification multiplied by d_p, which is our cedar particle diameter. This is basically the geometric image that we have studied in our optics, plus the ds square, and we call it the effective image diameter.

If you want to find the effective particle image diameter by projecting it back into the flow, we must divide it by the magnification to get the value of dE. So, let us solve a problem to gain a bit more insight into what we want to find and what we are talking about. And regarding the convolution technique, we won't be going into the depth of it, and you can study it from some literature available online. So, let us see; let us find this dE value for a particle diameter, say d_p, equal to 400 nanometers, using a 40x microscopic objective with a numerical aperture of 0.

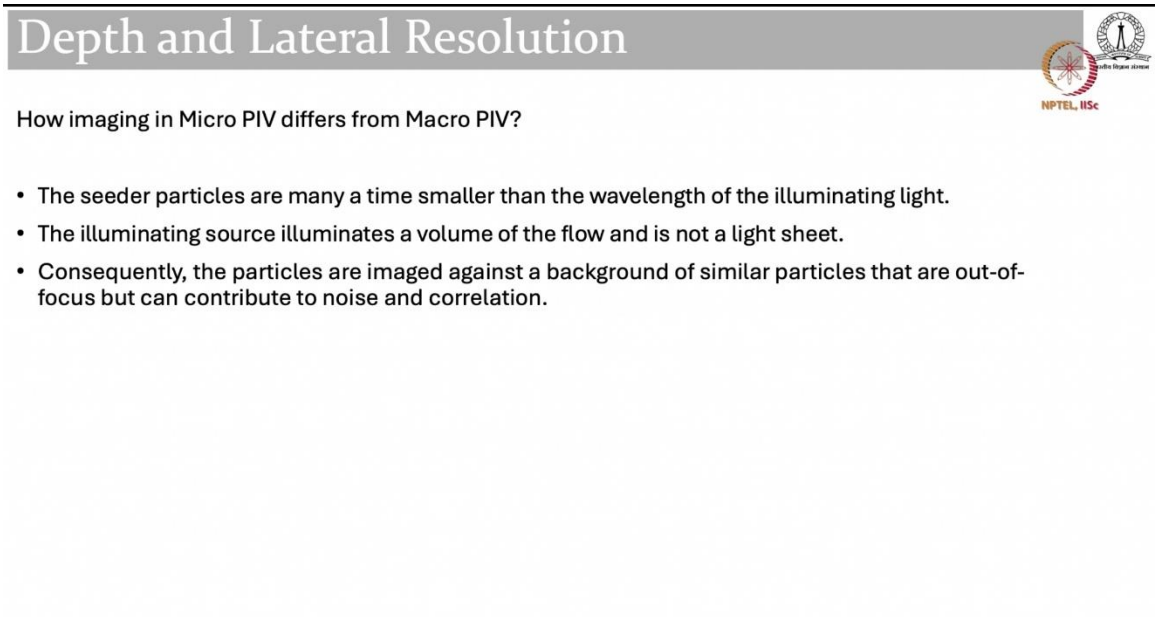
65, which is a high numerical aperture. And we must let us consider the light to be a green light source. So, let us find the ds value first, which is nothing but 2.44 times m times λ by 2 times na, and if we solve it, by putting the values like this, we should get something like 40 microns. So, basically, this is the point spread function that we talked about previously. Now let us see what the value of the geometric image is, which is given by m times the magnification factor times the particle diameter, and if you solve this, you get a value of 12 microns.

If you start finding the effective diameter using the formulas we talked about previously, we'll get the value of d effective as nothing but 41.76. It can be made more accurate, but just for understanding, I will tell this value. And you can see that ds dominates here compared to the geometric images. And you can change this domination by adjusting the numerical aperture and other factors, as we discussed previously.

So, finally, the value of dee will be nothing but 41.76 with the magnification factor that is around 1 micron. So, what we mean is that for this kind of optical system, where you are considering the particle size to be 400 nanometers, this should be 400 nanometers. So, just a second. No, sorry, I think this should be "let us consider it 300" because I have calculated for 300; you can see I have written 300.

So, this is fine. So, a 300-nanometer particle will look like a 1-micron particle, so you can see a particle on the order of nanometers starts looking like 1 micron. You see this error; there is a difference of around 700 nanometers in the diameter of the particle. This is basically the problem you face when you are observing the resolution in a single plane, and this is caused mostly by the diffraction of light. This is mostly unavoidable, but you can improve it, and you can take these calculations into account. Take into account while doing your post-processing, and that's how you can actually reduce the error in your output.

Additionally, using convolution, the uncertainty in displacement can also be obtained, and if you do that, you can see how to reduce the uncertainty as well. So, finally, I will briefly recapture what we have discussed in this resolution part because this is really important when you are talking about micro PIV, and this is the major part where you distinguish between the micro PIV and the micro PIV system. The cedar particles and those things are all fine, but if you are not able to resolve. Things well, then you won't be getting accurate results, and the method of resolving differs here a lot compared to the micro PIV system. So, basically, we'll try to answer the question of how this imaging system differs in the two systems.



The first thing is that we have already discussed these things, so the cedar particles are many times. Smaller than the wavelength of the illuminating light. So, as we have talked about this before, in the case of macro PIV systems, the cedar particles are almost on the order of 10 microns to 50 microns, and those are much larger than the wavelength of the illuminating light, normally a green light source. But then, in the case of micro PIV, you start going down to nanometer-level scales where the diameter becomes smaller than the wavelength of the light, and this is where this imaging system starts to make a difference, and you start using techniques like fluorescent imaging to view the particles. The other thing is that the illuminating source illuminates a volume of the flow and is not a light sheet, so basically, it's a very relative thing.

Basically, how you define a light sheet can again be considered a light sheet if you are seeing it in a much larger volume of the test section. So, basically, in a micro PIV system, we illuminate a volume of the flow and not a single plane. And unlike in the case of the macro PIV system, where we saw that the region of interest is of much larger depth, we can simply use a laser light of 1 mm to 2 mm thickness to resolve the particles in that region of interest. Another thing that is really important is that, as a consequence of using a volume illumination, the particles are imaged against the background of similar particles that are obviously out of focus but can significantly contribute to the noise and the correlation. So, as we discussed, I mean about the depth of correlation, which was, like, say, beyond the depth of correlation, the intensity of particles will be sufficiently low to contribute to the correlation, and consequently, it will not hamper your output much.

But your system should be designed in such a way that your images are taken with a background of similar particles, that they do not generate a lot of noise, and that they should not actually contribute to the correlation that gives you the ultimate velocity vectors.