Elementary Stereology for Quantitative Microscopy
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Lecture - 11
Basic Stereological Parameters

So this lecture, we will now come down to saw the stereological relationships that you have seen. But how do we get them?

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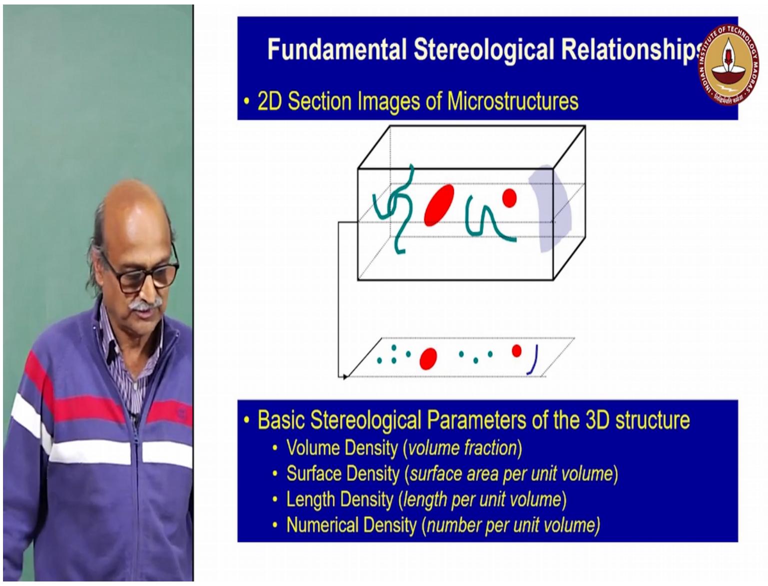

How do we derive them, that you would be see and; some of them that you have seen volume fraction of course, you have seen there is nothing much to derive I think it has been derived in some sense. But I will still show you a more rigorous derivation of how volume fraction is related to area fraction, then you have surface area per unit volume, how do we get it that. Length per unit volume, if I have linear elements, in a microstructure like this locations and if I have precipitate so I want to count the number of precipitates per unit volume.

So, numerical density so, these are the four relationship we will first see at.

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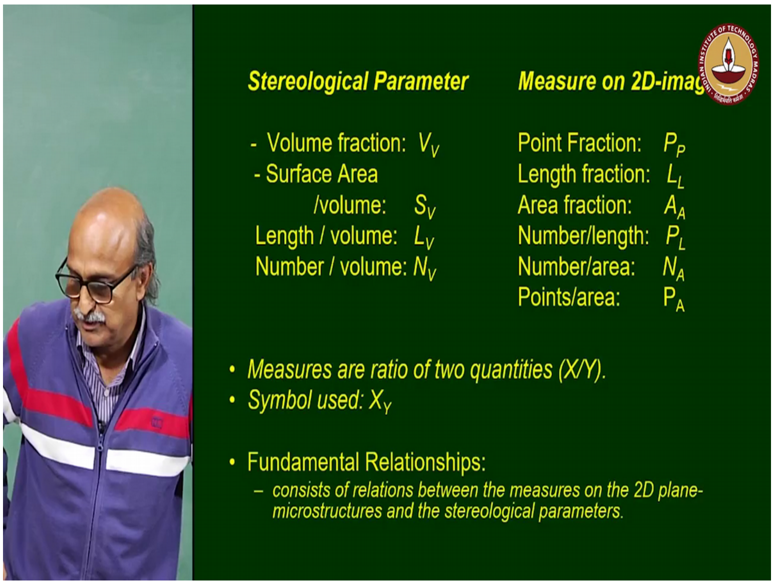

If you look at this slide this slide actually introduces some of the stuff that you have seen and, it kind of introduces a kind of symbols that one uses in stereology. So, on your left are those four fundamental parameters that we want to measure volume fraction, we use the symbol Vsub V, surface area per unit volume S sub V, length per unit volume L sub V, number per unit volume N sub V. On the right hand column are the measurements that one can make on a two dimensional microstructure.

So point fraction is 1 that you have already aware of length fraction. I will be introducing what it is, area fraction you are by now you are aware of P L number of intersections per unit length that also you are aware of the with the grid of lines, number per unit area. So, if you are having particles how many Particles that you see on a 2 dimensional image, in points per unit area. Where how do we get points on microstructure?.

Well if your linear elements in 3 dimensions you would get points, on a two dimensional image. So, the right hand are the measurements that you can make. And these measurements are then related to the left hand side are the parameters of the structure that we want to estimate. So, you will see that all of these measurements whether on the left hand side the parameters or the measurements on the right hand side on the 2 dimensional image are actually ratios of 2 quantities ok. X by X upon Y. So, for example, if I look at area of fraction it is area upon area, if I look at number per unit area it is number upon area, if on the left hand side I will look at surface area per unit volume. So, X is serve area and the denominator is volume.

And in general, this is the way we have defined the symbol as well X subscript Y to keep things simple. And we will now explore the relationships between the measurements made, and the parameters to estimate.

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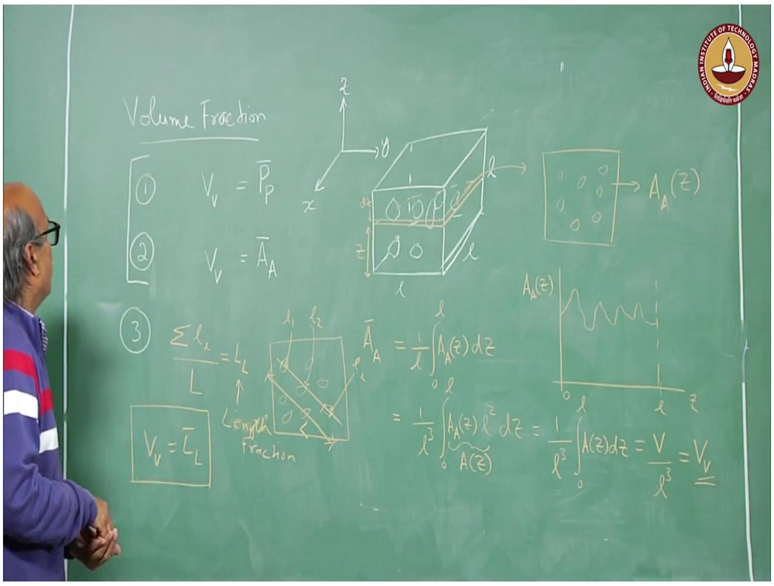

So, let us begin with volume fraction. Considering volume fraction you are already know, that if I measure point fraction on a microstructure, and make number of measurements get an average value of point fraction, then this becomes an estimate of the volume fraction, now another way of relating volume fraction. So, this is like 1, another way we can relate, volume fraction is through area fraction. I make measurements of area fraction on several images, get a average value of that and this becomes also an estimate of volume fraction.

Now, this is what I am going to now show, that this relationship we have already shown is true. Now, we will look at this relationship and show that this is also true directly without going through, point fraction. So, in all these cases, what I am going to take is a 3 dimensional space a cubic space the way I have been I have taken for many other situations. I will simply take up basically you can imagine it is a block of material ok, in the shape of a cube whose dimensions are l.

In this, I have the second phase particles. distributed inside the 3 dimensional structure and I take a section through it, I will take the horizontal so, let me also label the axis; so, that so, this is my x direction y direction and z direction, if I look at this, microstructure I will see, particles which are sectioned by this plane probe. Let the area fraction observed on this b A sub A and its it has been sliced at some random distance z.

So, that is why this is being referred to as area fractional position z. Now, imagine if I section this at different positions of z what I will get, if I were to plot all the way from 0 to l z varying from 0 to l, area fraction z obviously, at different sides I am go to get somewhat different values of area fraction and it may vary in some seemingly erratic manner ok.

Somewhere it will be higher, somewhere it will be lower and there will be some kind of an average, if I would have to add up all of these, then I will get an average area fraction. So, let us look at it this way; if I have to now find an average area fraction. What I can do? I can integrate this function, I can integrate this function from 0 to l A A z d z and here have to divide the centigral by l to get an average value.

This is of course, assuming that my all positions of z are equally likely, then only this a average, function works. Let us do a little bit of manipulation here, suppose I multiply and divide by l square; that is area of a single section. If I do this will become 1 upon l cube integral 0 to l; area fraction at position Z, times l square which I am keeping inside the integral all have done is I multiplied and divided by l square. Now, what is this quantity, A A z times l square is nothing, but the total area of these particles observed at positions z.

So, this could be then written as A z right, this is the area of fraction, then if this area of fraction is multiplied by the total area, then that gives you the total area of the particles. So, this becomes 1 upon l cube integral 0 to l A z d z. Now, this integral A z d z, if I look at just quantity A z d z this is the volume of the particles in a thin infinitesimals slices of thickness d z.

And what is happening in this integral, I am adding all of these thin slices infinitesimals slices all the way from 0 to l. What would that give me? This integral that will give me the total volume of the particles right so, this would be the total volume of the particles divided by the volume of my cubic space; this is nothing, but volume fraction.

So, this formally shows that this relationship is indeed correct that the area fraction is an estimate of volume fraction. Third possibility yes I take a microstructure, in which I am seeing these particles, if I superimpose on it a line, or a grid of lines it does not matter and, I measure these intercept lengths, so this could be l 1; out here the length intercepted between a particle, this could be l 2 and this could be l i and so on. All the particles that are that the line goes through you measure the length intersected within the particle ok.

If I sum all of these lengths which are intersected within the particles, divided by and you divide the sum by the total length of the line or lines ok. I can put a set of parallel lines and do this exercise. So, divide this by l that this is called just like you call area fraction, this we can call it as the lineal fraction, or length fraction I should say just call it length fraction, that the fraction of the length of this line; line intercepted between the particles from one boundary to another boundary.

This is also an estimate of volume fraction. Now, I am not going to prove this, but it is easy to prove you can prove it the way, this has been proved on a 2 dimensional section you can prove that length fraction is actually area fraction. And since we already prove that area fraction is volume fraction, with the relationship volume fraction is estimated by the length fraction becomes true.

So essentially I have put down 3 different ways in which we can measure volume fraction, this is this is relationship is generally not used for estimating volume fraction in materials. So, we will ignore this; and I want to look at these two method of measuring volume fraction, and I want to analyze I am good analyze it very briefly, I am going to show you kind of a statistical analysis,but in a very brief manner; that what would be the relative errors involved in estimating volume fraction by doing point count, by doing areal analysis. And then try to see, which is a better method of estimating volume fraction.

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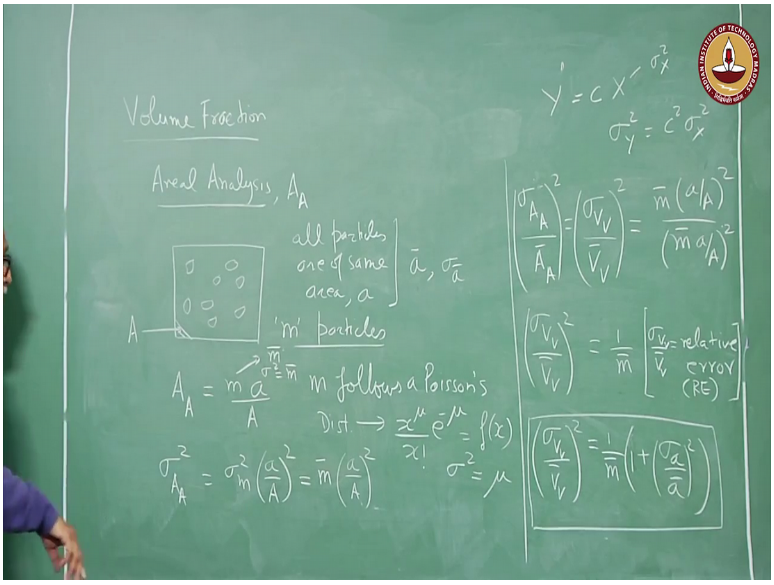

So, let us look at first areal analysis I am go to skip most of the steps in this statistical analysis because that is quite involved. but let us do the following that I have a microstructure, which consists of second phase particles.

Let us say that suppose we say that all these particles are of the same area ok. So, let us say all to begin with I if I assume that all particles are of same area a, and there are m such particles ok; and the total area of my field of view, or my microstructure is A capital A, then clearly the area of fraction would be total area of the particles divided by the area of my region over which I am measuring the area of the particles. So, the total area of the particles is simply m times a; and the field over which I have made the measurement is capital A so, my area fraction is simply m a m small a upon capital A.

But of course, now imagine that m here is a random variable. I will get some number of particles in one field of view I will get another number in another field of view and so on. And we have shown earlier in one of the lectures that; m can be m can be modeled as following a Poisson's distribution ok. So, m follows a Poisson's distribution; which is actually x to power mu upon x factorial e to power minus mu, this was the probability density function for the or rather not this a probability function for the Poisson Poisson's distribution which is a discrete distribution, where x takes up integer value. So, x x here is a same as m here ok.

So, I want to find out what is the variance; or the standard deviation sigma in my area fraction measurement; well I do know from the property of the Poisson distribution that; the variance sigma square is equal to the mean value mu. Now, suppose on an average my mean value of m is m bar ok, then the variance of m would be equal to the mean value which is m bar ok.

Now, coming back to this, what would be the variance then in area fraction. The variance in the area fraction is going to be and we use a property that if X is a random variable and, if it is multiplied by some coefficient C, then this gives me another random variable Y and if variance of X is sigma square X, then variance of Y is equal to C square sigma square X ok. This comes from this comes from what is known as propagation of variance from one random variable to another random variable. and this is a linear relationship so, this whole.

So, here what would happens C is my small a upon capital A so, my variance in this is sigma square m times a upon A and squared. Now, but I know that variance of m is nothing, but the mean value of m because; it is following a Poisson's distribution. So, this becomes m bar times small a upon capital A squared.

Then I can write; the variance in the measurement of area fraction, or the estimation of area fraction, I can let me divide this by the average value of the area fraction; squared, this is nothing, but variance in the volume fraction estimation divided by the average or the mean value of the volume fraction squared.

At this is simply equal to m bar a upon A squared divided by what is the average area of fraction, it would be m bar into small a upon capital A; m bar small a upon A and this whole thing has been squared so I need to square this. So what I will get from here, what I will get from here is small a upon capital A will get cancelled and this will be m bar and below would be m bar square,

So, this will become 1 upon m bar. That this sigma v upon v bar this represents kind of what I can call it as; relative error in my measurement ok, because sigma, volume fraction is nothing but the standard deviation so the dispersion in the volume fraction estimate, divided by the mean value of the volume fraction ok. So, this is it is relative to the mean value, you are normalized that with respect to the mean. And so this represents on the left hand side, then square of the relative error ok. And I will call this relative error is R E subsequently.

Now in this simple derivation, the error is simply related to the relative error simply related to how many particles you actually measure their areas ok; whether you are going to measure 100 particles, 200 particle, 500 particles, now is going to depend on what kind of relative error you can take right, can you take 10 percent error, or you can take 5 percent error; but this is only a approximate result I have produced here. In the sense what I started with I assume that all of these particles had the same area a, but we know that is not going to be true; there is going to be an average area of the particles, and there is going to be some kind of a standard deviation that would be there.

So there will be a, this there is go to be a distribution of areas that so therefore, the standard deviation in the area themselves in should also contribute to my error; and that rest of the analysis I am going to skip how I can get it, but it can be shown; that this is equal to 1 upon m bar times 1 plus sigma a upon a bar squared and the whole bracket closed. So, this is 1; for constant size particle sigma a would be 0 psi come back to the same relation, but then there is an added relative error, that has been added because of the distribution of areas of particles.

So this is an important relationship which we will see soon how you know what how many particles I should measure; in order to keep my relative error with in desired limits.

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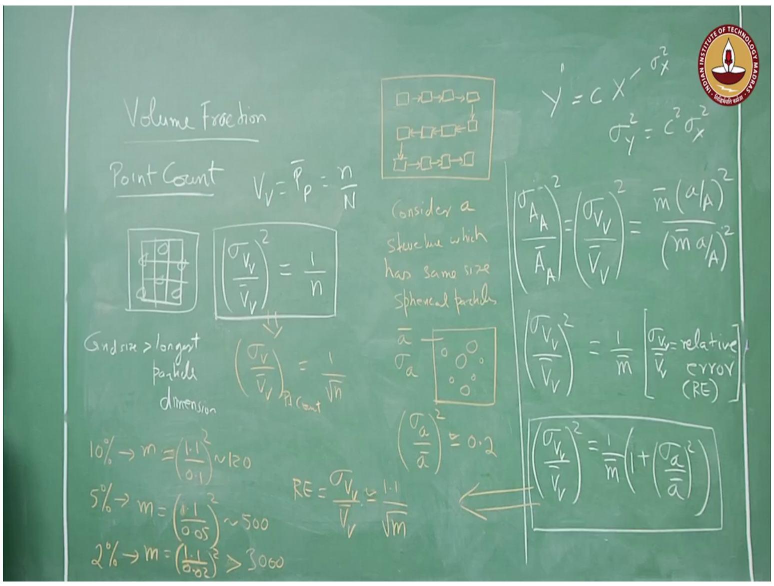

Now the same analysis you can do with point count ok. If I use a point count technique for estimating volume fraction; and I am going to skip that, that all the steps for the derivation because, that is even longer because there you will get Poisson distribution would have to be taken into account and you will also have to consider what is the probability of point falling inside the particle that which is going to follow a binomial distribution. To together, put together, gives me a very interesting result.

If I have a microstructure of part isolated particles again in which I put up point grid with the restriction that the grid size is larger than the longest dimension of the particle ok. So, the grid size; is greater than the longest particle dimension, then all it means, that only one point can fall inside one particle; that is where by putting this restriction; only one point falls inside the particle you can show; that the relative error squared in the estimate. Where the estimate is given by the point fraction which is equal to small n upon capital N, where small n is the number of points falling inside the particle capital N is the total number of points in the grid; this is simply equal to 1 upon small n ok.

Now let us compare these two results. And then decide which is the method I should use for estimating volume fraction, in order to do this, let us consider a structure which has same size is spherical particles; in the 3 dimensional structure I have the same size spherical particle. So, what I am going to see on the 2 dimensional structure, am I going to see same size circular particles, no I am going to see variable sized circular particles; depending on where the section cuts the spherical particles.

So this would have an a bar and standard deviation of the area, area of the particles. It can be shown that if my spherical particles are of the same size, then sigma a upon a bar squared is approximately 0.2 again we are not going to prove this ok, but this can be proved again from geometrical probability of cutting the particles.

So, I know for this if there was a distribution of the sizes in the spherical particles, then this variation is going to be even larger. So, this is the minimum possible variation you would get in the two dimensional microstructure hence, that relative error; can be written as can be approximately written as 1.1 upon square root of m, where m is the number of particles measured ok.

So I have to remove the square part so that I get directly relative error by measuring the areas ok. If I am doing an areal analysis, then this is going to be my relative error for constant size spherical particles. So, suppose I want let us say how many particles do you think I need to measure, if my I want to restrict my relative error to 10 percent; 10 percent means 0.1 here ok.

So, what do you think m would be of the order of m would be of the order of 1.1 upon 0.1, when m would be equal to squared and this would be approximately 120. So, I should measure areas of at least 120 particles just to restrict my relative error to 10 percent.

If I want to restrict it to 5 percent, I need to measure 1.1 upon 0.05 this times squared, and this would be of the order of 500 particles I need to measure. A let us say I want to be very accurate; keep it to 2 percent error, but I do not want more than 2 percent relative error, then m would be 1.1 upon 0.02 squared and this he would get a greater than 3000, I need to measure three thousand particles ok.

So I need to measure areas of 3000 particles, to keep my relative error within 2 percent to have to keep it within 5 percent I need to measure at least 500 particles. On the other hand by point count, I am going to be very similar because, this is this is going to be here for point count; for point count the relative error is going to be 1 upon square root of n which is very similar to 1.1 upon square root of n.

So, I am going to get very similar result, that if I have to keep my relative error within 5 percent I need to measure 5 areas of 500 particles; by point count also if I want to restrict my error within 5 percent, I need to do count of at or at least 500 point must fall inside the particles.

And I will simply have to count, which do you think would be a easier method to get the same relative error. If I told you that you take and start measuring the areas of each particle, get me area fraction that gives me estimate of volume fraction. And or use another method like point count and just count the number of particles to keep my relative error within 5 percent, which method is going to be which method you think would be easier to do, because in one case you have to do purely point count no area measurement is involved of individual particles.

Here I have to measure areas of 500 particles. In which case I will be covering a larger area, of my sample point count because to get 500 points inside the particles, I will have to cover a much larger area then this; but that may be alright because our samples are have even if I if my sample has large enough area, I can easily cover that and do counting much faster more efficiently with less chances of error versus measuring area. So, whenever I have to measure volume fraction manually, if that point count technique becomes the superior technique for estimating volume fraction.

Then I can use a grid which is very course.; so very quickly I can count and cover a large number of the large area in the sample, so point count becomes more efficient, but if I have a microstructure which is such that automated image analysis can perform this analysis then what if even area of fraction might be a better way to go, if a automated image analysis is possible. If it is not then almost always point count is going to be the superior technique; or the more efficient technique.

So, generally you should adopt a strategy for measuring volume fraction, so if I am one is you can take digital images, this is my sample. So, I should move the sample under the microscope in a systematic manner, so I can take one view here, second here, third here, fourth here, and so on. And then move down so, this is the way if we do this systematically instead of just here and there like that; do a systematic scan of the sample and take images like this and then put your grid on these images to estimate the volume fraction.

So you need to take large number of areas, if you want accurate results ok. So, this is important point to consider and this would be important point to consider for all other measurements as well ok. Are there any questions? I can answer who is ok. So, this completes my volume fraction estimation let us look at now those other estimates and, we need to show them how those stereological relationships get derived.