Elementary Stereology For Quantitative Microscopy
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Lecture – 01
Method of Stereology

So this is a course on Stereology and Quantitative Microscopy and the. I am going to introduce to you in this first lecture actually what is stereology. So, if you the term itself if you look at it goes back now more than 50 years back and why did it evolve, why did we require such a tech technique that we will be discussing or in characterizing microstructures and here the characterization of microstructure implies we are talking about geometrical characterization such as volume fraction of second phase.

It can be size of particles size distribution of particles it could be size or size distribution of grains it could be density of dislocations so many different quantifications that are geometrically possible with microstructures and we do know that the microstructures evolve as a result of the processing you do to the material and that in turn governs the properties of the materials and that is why there is a requirement for characterization of these structures for example, volume fraction is going to affect perhaps the strength property that ductility grain size for example, can again impact mechanical properties and so on.

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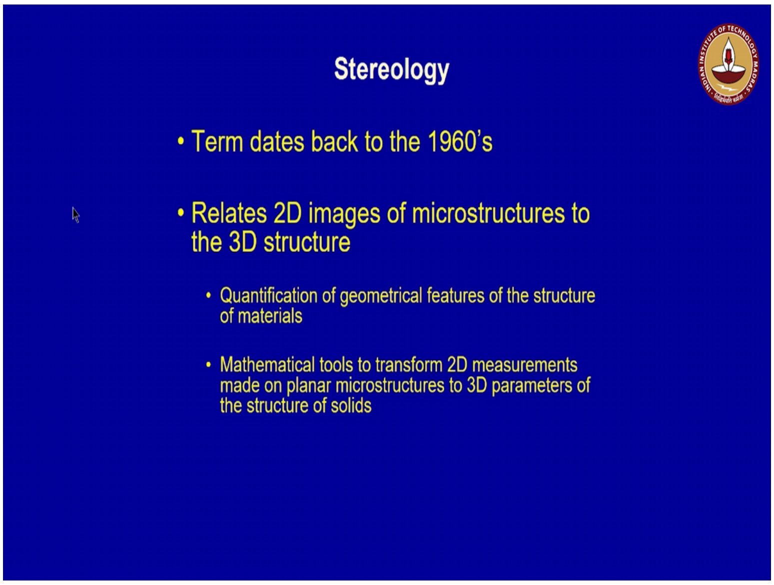

So, the term stereology dates back to the 1960s and what stereology attempts to do, it is to relate 2 dimensional images see you when you look at a microstructure it is a 2 dimensional image the structure we have is actually 3 dimensional. So, how do I relate a 2 dimensional image to the 3 dimensional structure for example, volume fraction is a 3 dimensional quantity, but you are only looking at a 2 dimensional image even size of a particle is a 3 dimensional quantity, but you are again looking at a 2 dimensional image and this brings in some challenges as to how you can relate it what you observe and measure on a 2 dimensional microstructure to what parameter in the 3 dimensional microstructure.

So, again quantification of geometrical features of structure of the structure is what stereology is about and in ordered to do it consists of many mathematical tools which enable you to transform 2 dimensional measurements you measure on a plane microstructure to 3 dimensional parameter of the structure and some of the 3 dimensional parameter as I said could be volume fraction size, size distribution and so on.

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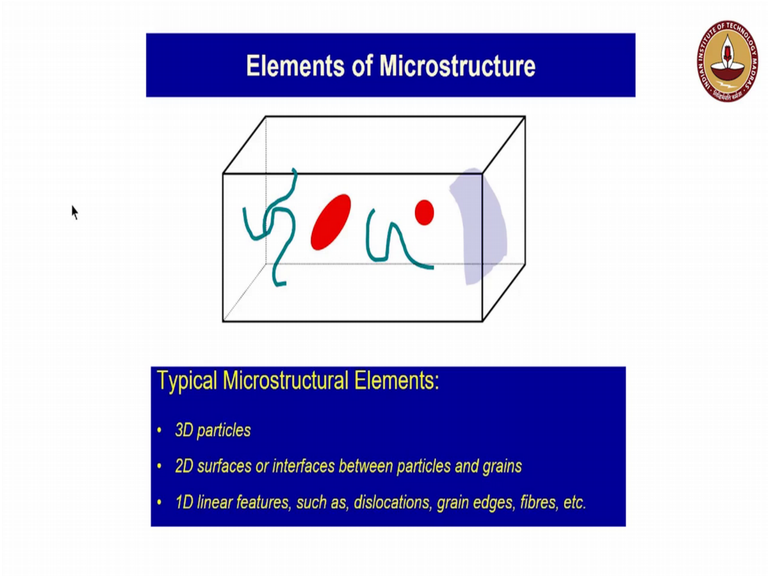

So, before we proceed into the geometrical characterization let us look at what are the geometrical elements which makeup a microstructure, I can isolate those elements for example, if you look at a block of material in which what are the constituents, constituents could be linear elements like this locations, like triple edges, it could be surfaces like grain boundary surfaces or it could be solid particles. So, I have these various features 3 D particles, 2 D surfaces or interfaces and 1 dimensional linear fake features such as dislocations, grain edges, fibres in a composite material.

Now, what do I do in order to, but our material is opaque generally most of the materials that we as material scientist deal with are opaque materials. So, we cannot see so, in order to observe these the structure in the material what do I do? I actually take a section through the material there is the simplest way of doing it polish it and put it under sub microscope, it could be an optical microscope or a scanning electron microscope and observe the structure and I have to of course, prepare the surface. So, I polish the surface and then etch it and so on.

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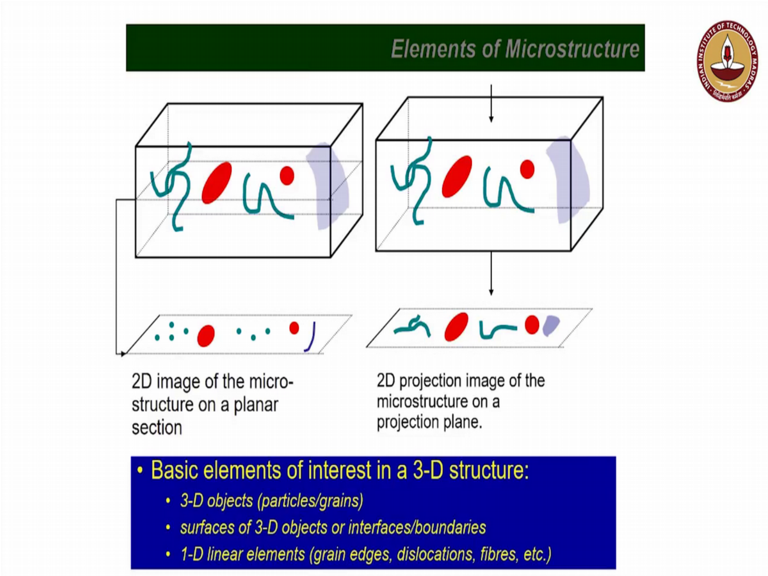

Here is what we are actually doing that, one is taking this section through the 3 dimensional block and on that section I observe it under some microscope. So, what will I see, if there are linear or line features they will observe they will you will get them as points on the section is not it? If a section cuts a line you will get a point, if a section cuts a boundary a surface you will get a line and if a section cuts a 3 dimensional solid particle you will get a section of that particle which would be a 2 dimensional profile this is one way of observing a microstructure like this.

Another way we observe microstructure if you think back is producing a projected image which is possible if my material was transparent light, if this material was transparent to light then I will get lines will be projected as line surfaces will be projected as area elements and solid particles will also be projected as area elements forming a projected image for biologists to understand this is very straight forward because the use a transmitted light microscopy because their tissues or that they are observing are generally transparent to light. So, they are able to get a projected image even as material scientist when we use a transmission electron microscope we project the structure on to a 2 dimensional plane.

So, these are 2 different ways of observing the structure one is a section image and other is a projected image and you can clearly see that these 2 images are very different, what you would observe this points on a section image you would get lines on a projected image, both of these structures both of these 2 dimensional images can be quantified and then related to the 3 dimensional structure, but the stereology behind them can be quite different as to how to do this transformation. So, in these set of lectures we would deal only with the planar section will not be dealing with the stereology of projected images in this set of lecture.

So, just to summarize basic elements of interest are 3 dimensional objects surfaces of 3 dimensional objects or interfaces or boundaries of grains of 1 dimensional linear elements, grain edges, dislocations, fibres, etcetera.

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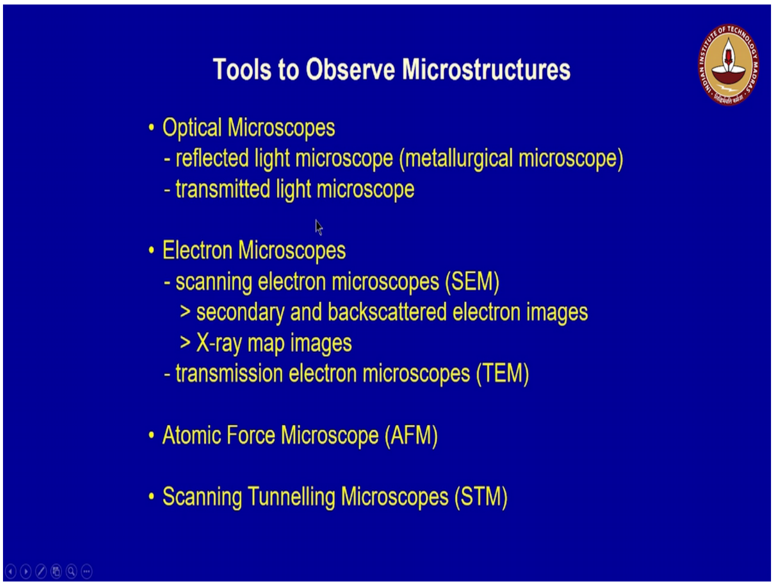

Now, let us have a look at some real microstructures and the tools that we used to observe this microstructures I am sure that when you most of you would be aware of the optical microscope, whether it is a reflected light microscope, which is what we call is the metallurgical microscope for opaque materials and then you have the transmitted light microscope, where the light goes through the sample, which means the sample has to be transparent to light, going to electron microscopes you have scanning electron microscopes, where you can get secondary of backscattered electron images, even X-ray map images or going to transmission electron microscope which becomes again a projected image time if you are using transmission electron microscopy. Then you have plethora of other mi microscopy tools such as atomic force microscopes scanning, scanning tunneling microscope etcetera.

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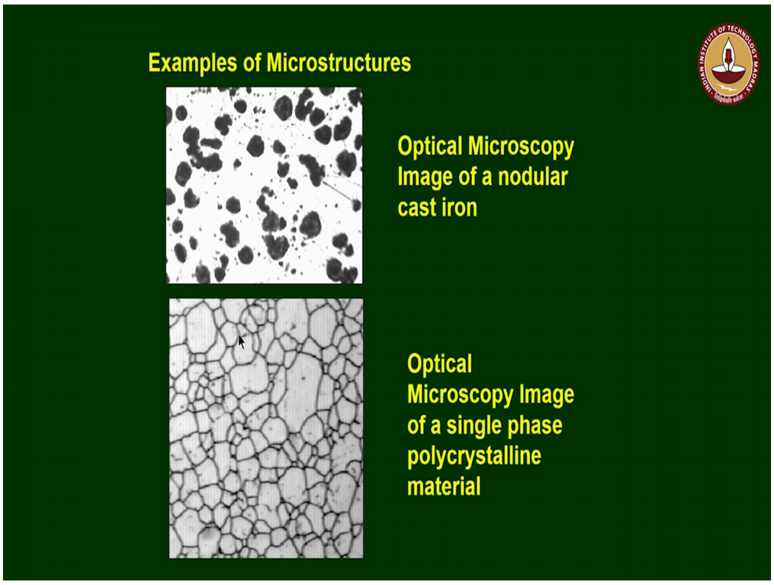

All of these will give me 2 dimensional images here is some examples this is what you would observe in a typical nodular cast iron put under a reflected light microscope or a metallurgical microscope this is a single phase polycrystalline material where you can see the grains and you see this black lines which are the grain boundaries which in the 3 dimensional structure are actually surfaces.

So, what you see here for example, these lines meeting at 1.0 and this typically you will see that grains are meeting at 1.3 grains are meeting at 1.0 or 3 grain boundaries are meeting at 1.0 we should call as the triple point. In the 3 dimensional structure this triple point is actually a line which would be a triple edge with 3 grain boundary planes or meeting forming a linear element. So, if I look at these 2 microstructures what I am seeing, I am seeing section profiles of the graphite nodules which are actually 3 dimensional solid objects inside the material in this poly crystal image, I am seeing lines which are actually surfaces, I am seeing this triple points which are actually lines ok. So, these are the so, you should you are able to isolate the various geometrical elements of a structure.

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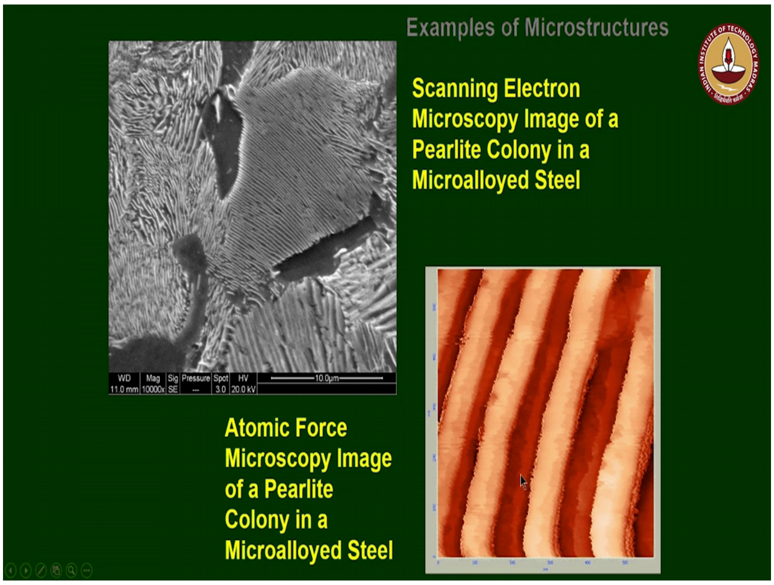

Here is microstructure observed in a scanning electron microscope of a ferrite pearlite structure of plane carbon steel. So, here actually the pearlite colony is shown prominently where you have alternate lamellae of ferrite and cementite. Now, one of the things that one is interested in which also control the property of steel is, how fine this lamellae are, what is the interlamellar spacing. Now we will see that stereology can be used to quantify such a parameter like the, what is called as a true interlamellar spacing.

The interlamellar spacing that you see on a 2 dimensional image is not the true spacing, the true spacing is a perpendicular distance between the planes of ferrite and cementite that are existing what you are seeing a only a section profile of these planes and depending at what angle the section cuts the spacing will be different on a 2 dimensional image. So, how can I convert a measurement with the where the spacing is not the true spacing to actually to the true spacing. So, we will see how stereology will help us to do that, this is simply a more magnified image of a pearlite using a atomic force microscope.

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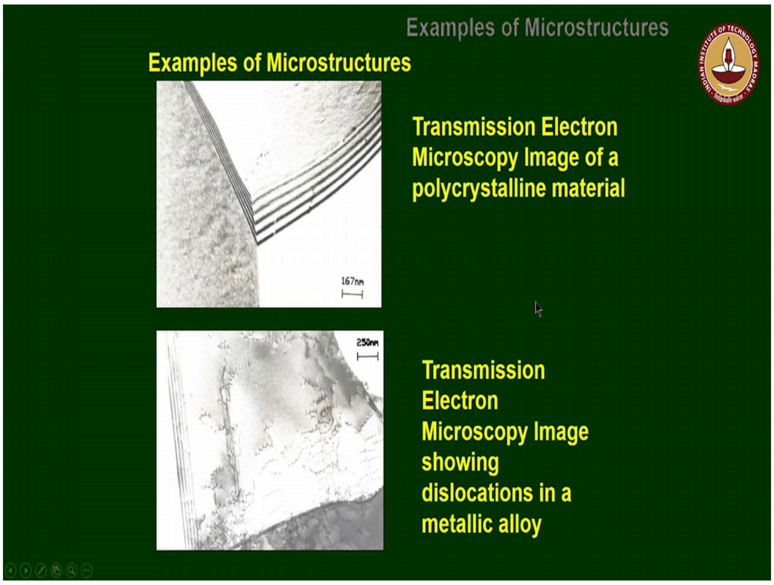

Here is a couple of projected images now in a transmission electron microscope so, here you are seeing basically again this is a single phase polycrystalline material in which this top images you see 3 distinct grains and 3 grain boundary planes meeting at a triple edge ok. So, this is a projected image where you can make out where triple edge is and the image below shows linear elements which are projected and what are these linear elements, these linear elements are the dislocations in the material. So, projection is preserving the line as line in the image.

So, now we come to a important concept of stereology which is sectioning of a material ok, generally sectioning we can cut section 3 dimensional block with the 2 dimensional plane if or we can also section using a line or we can section using a point. Now what does this mean? All of these sectioning processes also are termed as sampling of the material and sampling by probes of various dimensions. If I take a 2 dimensional section the probe is 2 dimensional, if I section it with the line the probe is 1 dimensional, if a section it we the point the probe is 0 dimensional.

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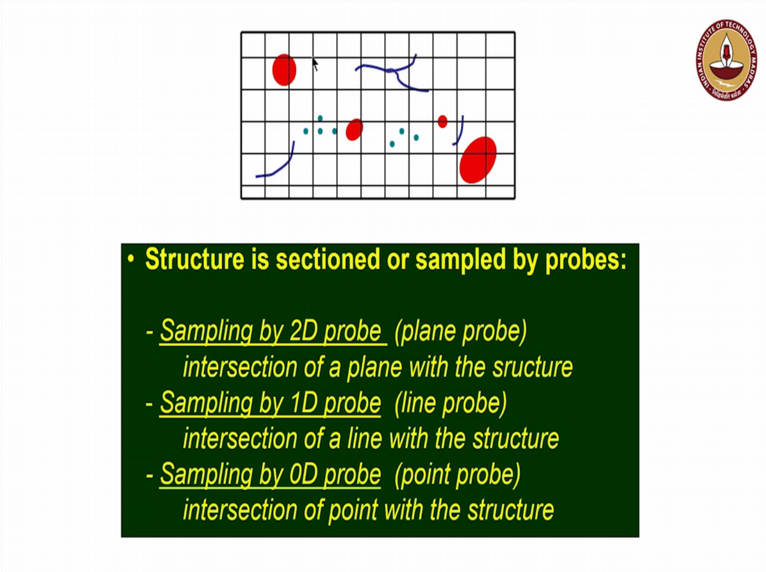

Now, to understand this sampling by a 2 dimensional probe or a plane probe is essentially nothing, but intersection of a plane with the 3 dimensional structure. So, when I intersect the 3 dimensional structure with a plane then I see that lines will appear where surfaces were there, points will appear where line elements were there in the 3 dimensional structure and area elements or section profiles will appear where particles were present in the material. So, this is called sampling by a 2 dimensional probe.

Now, what is sampling by a 1 dimensional probe or a line well, on this 2 dimensional probe if I put for example, this grid of lines this horizontal and vertical lines then this is sampling the structure by this lines. These lines then are called as simply a line probe and if you take any one particular line for example, if you take this line then one can look at the intersection that the line makes with the features with the geometrical features of the structure ok.

So, for example, this line intersects this particle I can look at this intersection by maybe intersection with the boundary of the particle or I can look at it as a line intersection with the solid particle itself and then I can look at the length that this line makes within the particle, I can look at the intersection of the line with all with this what you are seeing is, line is actually surfaces in 3 dimensions. So, I can look at the line intersection with the surface so, this is called sampling by a line probe.

Then finally, sampling by a 0 dimensional probe or the point probe, the point probe if I look at the intersection of these horizontal and vertical lines they intersect at a point. So, I can consider this intersection is points and these are all the point probes ok. This is a point probe, this is a point probe and this is a point probe, this is a point probe, now again just like with the plane probe and a line probe I can look at intersection of these points with the structure for example, I am only interested in looking at the how many points are going to intersect the particles. So, this is one intersection with the particle, this is another intersection with the particle, this is another intersection with the particle.

So, I am use this probes in order to understand the structure we will see this as the stereology unfolds here, that what exactly are, we meaning when we are sampling with these probes, but one thing to realize is this sampling word is used in a statistical concept which I will be coming later. So, let us continue with this inter interaction of the probes with the structure ok.

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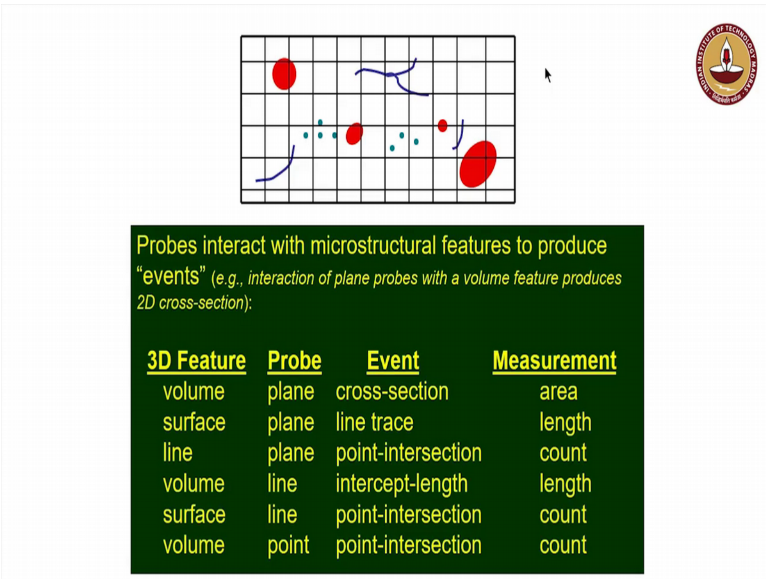

So, if my 3 dimensional feature here in this column, if it is a volume feature like a solid particle and if there is a plane probe, if this plane probe intersects the 3 dimensional feature then there is an event there is produced an event could be the cross section area or simply the cross section and the measurement we can make of that on of that event is the area this is one possible measurement one can make ok. There are other measurement of course, I can also measure the perimeter for that matter if it is a surface feature and the plane pro and we are using a plane probe then the event is a line trace and one of the measurements that we can make is the length of the sectioned line.

If you take a line feature in the 3 dimensional probe cutting with the plane probe you will get a point intersection right line with the plane you will get a point intersection, how many such points are there you can count them so, how many such events are there you can count them. Similarly, volume feature again and line probe is used you would get a intercept length the length within the line probe passing through the 3 dimension feature and the length and the and the intercepted length within the volume feature and you can make a length measurement.

Similarly a surface feature intersecting with the line probe you will get a point intersection and such points can be counted, volume feature with the point probe you would get a point intersection and again all so, all such points can be counted. So, this are the various combination of these probes one probe that I did not mention because if you look at all of this probes we have looked at 2 dimensional probe, 1 dimensional probe, 0 dimensional probe, all of these probes I have a dimension which is lower than the dimension of the microstructure this is a key point to note.

There is one more probe which is actually a volume probe which is called the dissector we will briefly look at that particular probe later on in this set of lectures and this word event that I am using this is coming again from a concept of probability that when we would be talking about, what is called as geometrical probability that if an event occurs in certain probability of that event.

So, for example, if a 2 dimensional probe a plane probe intersects a particle there is a probability of intersection of that plane probe with that particle what is the probability. We will have a look at that because all the stereologies then built up on such concepts like the geometrical probability where if there are let us large number of particles and there is a plane probe which cuts the structure not all the particles will be cut only some of them will be cut, how many of them will be cut would depend on what is the probability of the plane probe intersecting the particles.

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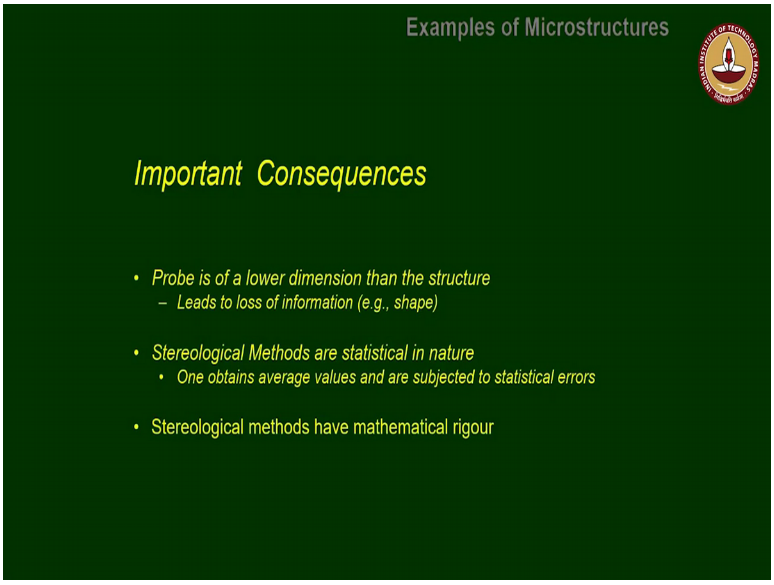

So, important consequences one as I said probe is of a lower dimension than the structure and a consequence of that is that it leads to loss of information for example, suppose I see on a microstructure elliptical shapes, what is the shape of the of such particles in the 3 dimensional structure? I cannot be very sure it could be coming from ellipsoidal particles, but it could also come from cylindrical particles that would also produce many such ellipses.

So, because the probe is of a lower dimension the information that we lose a shape so many times in order to analyze we have to assume certain shapes in order to go further with the analysis. Since probability is strongly linked with the stereological methods stereological methods are going to be of a statistical in nature and just like when you collect data statistical data one obtains average values and these average values are subjected to random or statistical errors and we will take couple of examples where we will see that how we can deal with these errors, how we can compute these errors or random errors that are there or the statistical errors that are there and third stereological methods have mathematical rigour ok.

So, they are based on a very strong footing of probability and statistics as we will see in this before even try to explore these the foundations of stereology let us take straight away an example of method of stereology. The 3 structures that you are seeing these are actually hypothetical structures, but they may be coming from spherical particles in 3 dimensions and they have been sectioned by plane probe so, you will get circular profiles of varying diameters.

Now, suppose I wanted to find even if I forget about what the 3 dimensional structure is suppose in this I wanted to find what is the area of fraction of these particles.

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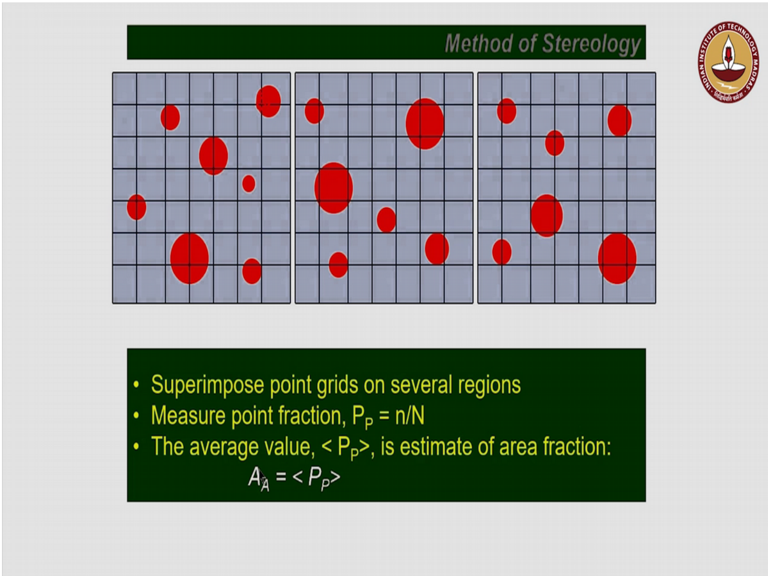

That is what is the total area of a particle in a unit area of the image of course, one can do this directly also that simply since these are circular particles I can simply measure the diameter and then of each particle and then I calculate what is the total area occupied by this particles, that area divided by the total area of the image in which these particles are distributed what give me the area of fraction, but if I dealing with very large number of particles which we have to in order to get a good estimate because your microstructures are going to vary from place to place in a statistical manner.

So, just one small region will not give you a very accurate estimates I have to take several areas and hence it may be very tedious. If I have to do this manually and then there going to be also my manual errors, that I will make and so on of course, I can do this with automated image analysis, but not all microstructures we can use automated analysis as well.

So, for timing being let us just consider just this 2 dimensional images and I have to calculate what is the area of fraction from all of these 3 structures and take an average value. So, I will present a stereological method to do this which should be very fast and which would we would be doing, you will be doing in this class shortly yourself put a grid of points on these images. Now this grid of points are again the intersections of the horizontal and the vertical lines, see I could have put isolated points, but those points are difficult to see and that is why we put a grid of points so, that this is a point, this is a point, this is a point, this is a point and so on.

So, if you look at this there are total of the 64 points and each grid so, and then the event that I am looking at. So, this is a point probe that I have applied and the event is out of those 64 points how many points are falling inside the circular particles ok. So, for example, this is a point here so, this is a point that falls inside the circular particle, this is another point which falls inside the circular particle, this is third point that falls inside the third circular particles and I calculate what is called as a point fraction, small n here represents a number of points that intersect the particles. So, small n represents those kind of events divided by the total number of points I have in the grid in this case 64 points.

Now, I get a point fraction value from each of the 3 microstructures and I calculate an average value of point fraction which I have represented in triangular brackets to represent the average and this average point fraction is an estimate direct estimate of the area fraction. So, this is a stereological method which would give me an estimate of the a area fraction as again with a statistical or random error associated with it which we will see also to calculate that random error.

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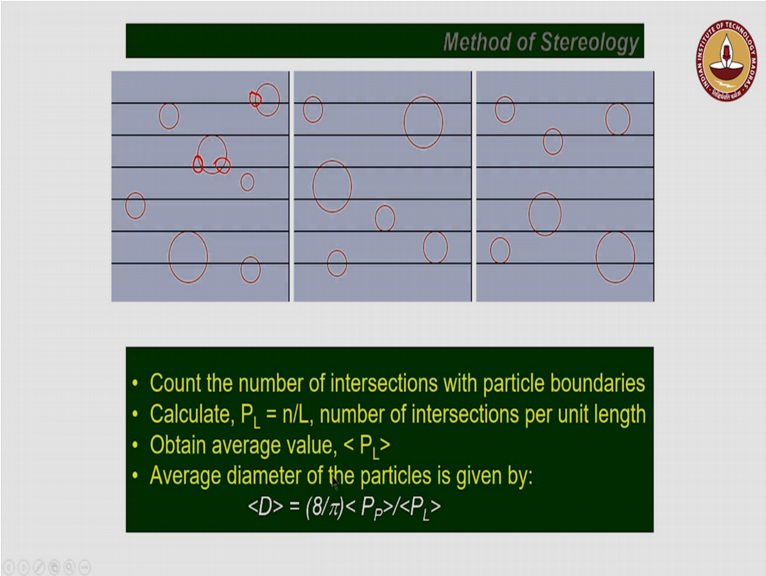

Now, this particular microstruct set of micro is the same particles, but now I am focusing on the boundary of the particles the interface interfaces of the particles ok. So, this is this is the phase boundary between the particle and the matrix and I again put a grid, but this time I remove the vertical lines I will just keep the horizontal lines and I count the number of intersections these horizontal lines make with the boundary. So, for instance this is a intersection, this is a another intersection, this is a another intersection and so on.

So, you count this intersection, you count this intersection, as well you count this one, this one, this one, this one, you total of all the intersections that are there I just highlighted only 3 of them and then let that be small n in a particular image in this image I will get some small n number of intersection I divide that by the total length of these horizontal lines ok. So, I measure the length of one of the horizontal lines and then add up the length of all of them put together, this is what we call as the number of intersections per unit length and the symbol that I am using is P sub L.

Using this get an average P L from all 3 images in this case and then I present another relationship whose foundations are based in stereology, that the average diameter of these particles in the 2 dimensional image is the ratio of point fraction that we have calculated in the previous slide divided by the number of intersections per unit length P L is calculated here this ratio multiplied by a factor of 8 upon pi gives me the average diameter of the particles ok. So, these are 2 relationships I presented and what we can do next is actually perform this analysis on a real microstructure ok. So, I will close this lecture here and we can look in the next lecture where these relationships are applying.