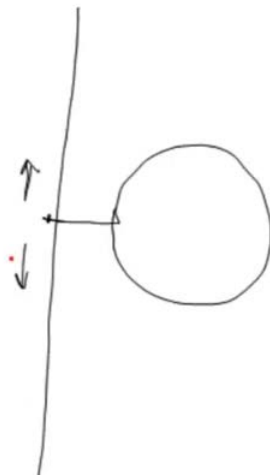


**Analytical Technologies in Biotechnology**  
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**Indian Institute of Technology, Roorkee**

**Module – 1**  
**Microscopy**  
**Lecture - 2**  
**Basic concepts in microscopy 2**

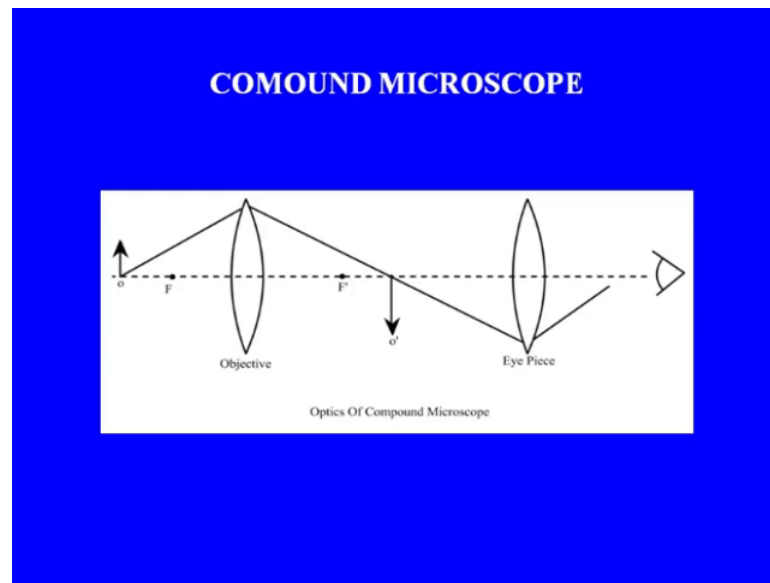
In this lecture, we are going to discuss in detail the optics of a compound microscope. Now, a compound microscope unlike a simple microscope has two lens system. Now, if you compare a simple lens it is made of a single lens, which can be hold or which can be in an object holder and could be mounted in a support. For example, if I show you in this screen.

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Then you can have a simple holder, which can be holding this lens and this lens could be moved up and down here to which can move up, and down to adjust the focus and magnification can be done on this lens. Now, as we see that compound microscope is little different from here, as I will show u here.

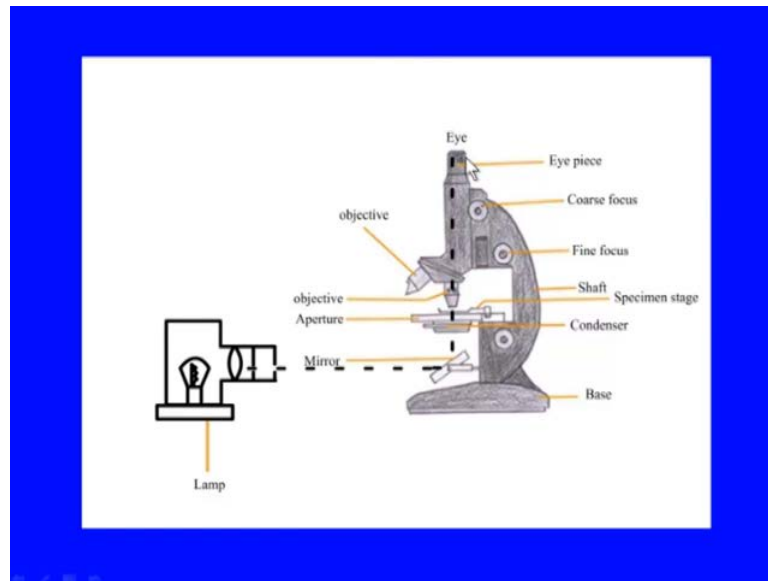
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Now, in compound microscope rather than one there are usually two magnifying systems one ((Refer Time: 01.33)), one defined by objective and other defined by the eye piece. Now, this avoids practical problem of making a lens of a very short focal length. If you see in this figure, the object is placed just after the focal length or more than the one focal length of the lens. The objective forms the image, which is real inverted image if you could recall we have discussed this in the last lecture.

Now, this real inverted image will act as an object for the eye piece and the eye piece will form another image, which will act as the object for the eyes. And finally, the image enlarged image will be formed on the retina of the eyes, which will be seen as virtual image or which will be seen as inverted image as we will discuss in later. Now, compound microscope, if you see this it is a very simple diagram of a compound microscope and we will discuss of all different parts of compound microscope here.

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The compound microscopes can be of different kinds they could be simple with different optics like fluorescence optics, it could be phase contrast microscope, it could be a stereo microscope or it could be even an inverted microscope and like so on. Now, here if you see as we go along between different components of microscope. First thing is the light source, which is uniform bright light source is placed either externally like shown here or it could be mounted in the base of the microscope right below here.

Now, there is another lens system, condensed lens system, which collects the light from the light source. And this is ah the condensed light passing from the condenser will be illuminating the object in a very small cone of light, which is focused on a very small area. And all stray light or extra light is being avoided by the apertures and the Iris, which are placed at different places.

The object, which is illuminated here, which is at the specimen stage will the light or the light from condenser will be illuminating the object and the light from the object or the specimen will pass onto the objective lens. Now, there will be two kinds of lights here, one, which is not altered by specimen and another, will be altered by the specimen. Now, one which is not altered that is not diffracted will be forming, the will be forming, which is not altered will be forming the background. One which is diffracted will be focused by the objective to form the final image at the intermediate image play.

Now, after objective there is a tube here, which connects the objective to the eye piece? The objective forms, which will be real and inverted image because the specimen is placed just one focal length away little farther from one focal length from the ah specimen. So, a real inverted image is formed in here, the inverted image formed acts as an object and the eye piece, which is connected here with another end of the mechanical tube will be forming, will be making image at virtual image, which is like here.

If you have recalled that when you put the object below one focal length then virtual image is formed, which is formed at the base of the microscope or at the mechanical tube and this will be acting, this will be acting as an object for the eyes. And eyes will be the ah eye lens will form the final image on the retina, and which will be viewed on the retina as in enlarged or magnified image because it occupies larger area on the eyes.

Now, let us discuss some of the parts here to understand the microscope. Now, here the condenser lens, which is will be discussing in detail also condenser lenses forms a very fine focused light onto the, onto the specimen, which is to avoid any stray light or, or light, which could form diffused image. Now, always there is a diffusing screen port before the light source, after the light source and before the condenser, so that the image of light sources is not formed here.

If you see here, objectives here, two objectives there are these objective lenses, which are all corrected. I could ah if you can recall all the lenses including condenser, objective eye piece are corrected for various aberrations. Now, here if you are seeing these two lenses, which are objective lenses here, more than two lenses could be put in, which could go up to five. Now, these objective lenses are attached to a rotating nose piece, which is threaded in here. And this is connected a tube length, which is finite tube length and on the other end of the tube end is the eye piece.

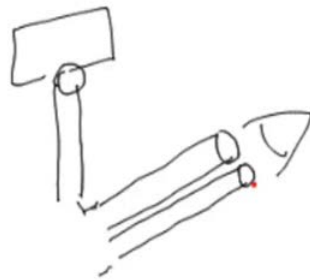
Now, here these objectives could be this nose piece could be rotated and the objective of different magnification it could be 10 x, it could 20 x, it could be 40 x or 60 x magnification objectives. It could be used for magnification as per the application and most of the time most of the advanced microscope has will, will be power focal or you can in other words. The specimen will not lose focus when you are changing the objective by rotating the nose piece, so that is the very important thing in here.

Now, mechanical tube length is fixed tube length and the problem there two kinds of optical system here, one is finite optical system as I will show you on the next slide and other is infinity optical system. It is very difficult to put in auxiliary components in finite optical system or fixed tube length because of problems of correct image formation, or sharp image formation due to aberrations and the problems. But infinity corrected optics there will be an option to put in the auxiliary components like, prism or polarizer or other things, so that you can be ah and without any problems in the image here.

Now, here if you see the objective the mechanical tube length will be defined from the nose pieces where it is threaded till eye piece is attached, Now, here if you see, if you consider eye pieces there could be different arrangements here it could be monocular, it could be binocular or it could be trinocular port. Monocular means, a single pair of a single pair of eye pieces attached in here. Binocular means, two eye pieces are attached and Trinocular means, the three eye pieces are attached in here. Now, eyes pieces could be arranged in different fashion.

Now, here it could be monocular or binocular eye pieces or a trinocular eye piece or we also call it as a trinocular port. Now, most of the microscopes will have an inclined viewing for an easy viewing, a direct viewing here through eyes. If you have an upright, upright eye pieces then it is very hard to view like, you have to stand and you cannot be easily viewing that. So, for inclined viewing like, I will show you on this screen the eye pieces or the mechanical tube lens are inclined at an angle, so that you can easily see the you can easily observe the image in the compound microscope.

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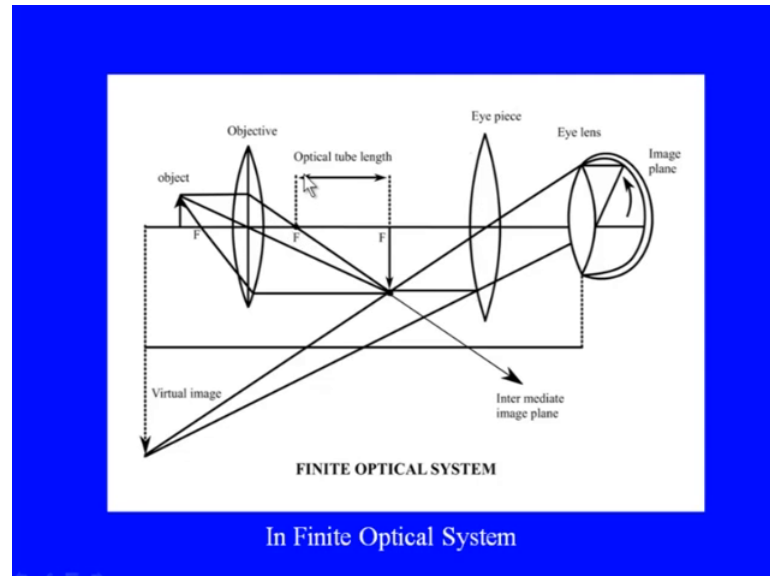
So, if I can say if I can binocular or a trinocular port then you will have a tilted eye pieces or in some cases the mechanical tube also might be tilted. So, you have a tilted ah binocular eye pieces and you will have another port, which will be for a trinocular port. So, what you have is rather than having one and you can see here, very easily in this. Now, binocular eye pieces the light will be from objective the rays, which are the rays, which are coming from the objective will be splitted by splitted and then enters the two eye pieces.

The trinocular port where you have three eye pieces there could be used for different purpose. For example, the two persons who would lie to view the object and then would like to view the image simultaneously. Then like for example, student and teacher like, teacher is either examining the student or the teacher is explaining a particular sample or a particular specimen to the student. Then a trinocular port could be utilized, another thing is that you can attach here, a camera or a screen to permanently record the images. And that is why you can use a trinocular port upright here, straight and your other part is inclined for better viewing or for a convenient viewing in here.

So, that is a very important part here, though we have made this figure in that way but you have is you have mostly inclined view in this. Now, we will discuss like there is closed focus, there is fine focus and these will be this is like, you can adjust microscope accordingly for focusing on to the specimen. Now, let us move on to the as we were

talking about two optical systems in the fixed tube length. Remember fixed tube length is almost around 160 millimeter as accepted widely accepted.

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Now, here in this figure, if you see I have shown you finite optional system. Now, finite optical system consists of objective lens, eye piece lens and then finally, you are viewing it directly through your eyes. What happens in finite system or finite optical system? Which is also we called fixed tube length. The object is placed little farther from the focal length like, you can see here, there is an object in the form of arrow as been placed. Now, this light it is illuminated object the light falls on the objective lens and it is focused at the intermediate image plane.

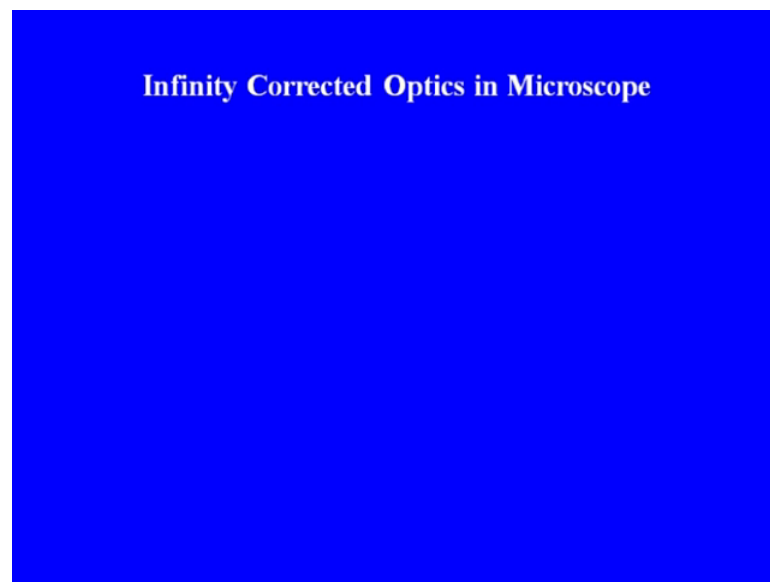
Now, this intermediate image plane will be placed in such a way that it is, it is this is little less than focal length of the eye piece. Now, if you see here, the optical tube length, which we were talking about the mechanical tube length or the optical tube length is from where the objective is threaded. And it's like, focal length of the objective on the other side, and till the intermediate image plane this distance is called optical tube length. Now, this acts as an object for the eye piece and eye piece will make a virtual erect image on the same side of the lens that is eye piece.

Now, this image is formed at a 25 centimeter or 250 millimeter, which is you can say a relaxed position for the eyes to view the objects. Now, this virtual image acts as the object for the eye lens and finally, an inverted real image is formed on the retina of the

eye. Now, which is of course, not perceived as inverted image rather it is perceived as erect image as it is here, by the brain. As we know that when we see the object directly from the eyes though inverted image is formed on the retina, but we see people as upright and erect.

So, infinite optical system it is very difficult to introduce auxiliary components between the objective, and the eye piece because there will be problems of spherical or other aberrations and the image formation will not be sharp. So, those to avoid these things and many time you might have to increase the tube length also mechanical tube length, which might not be possible.

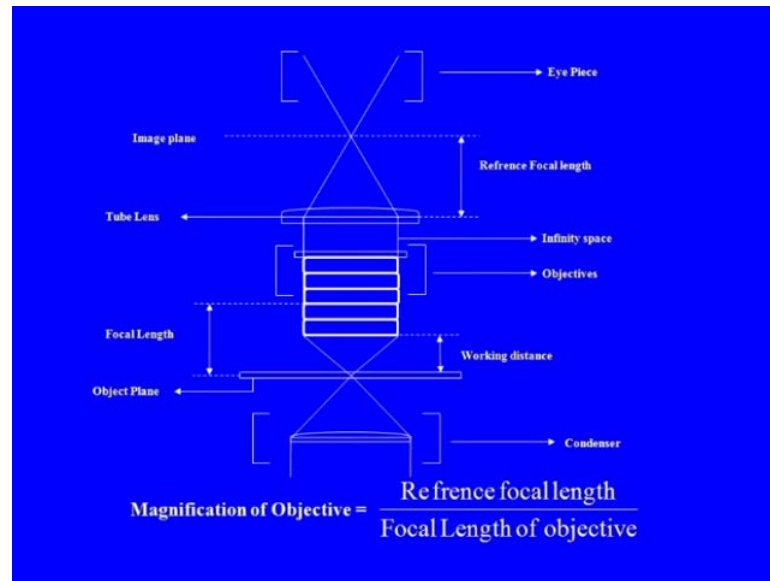
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So, what, what advance microscope now? Most of them contains this is an infinity corrected optics. Now, in infinity corrected optics what happens is that? You are able to insert optical components like, lot of polarizer and prisms are included here and without any image problem.



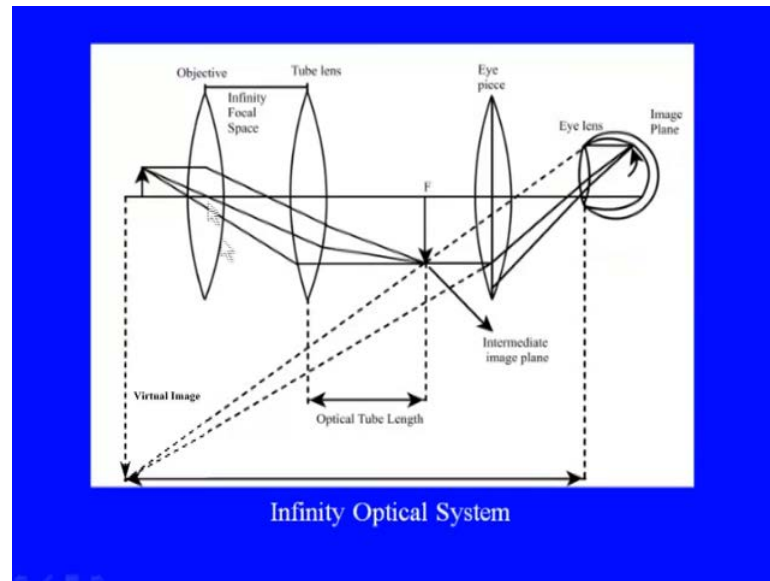
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Now, how does it work? In this simple schematic here, shows that what you have is? You have other lenses condenser lens system object plane. And what you have is? Objective lens, which is placed in here, there is an additional lens near to the objective lens and it is called tube lens. Now, what you have here? The light coming from the specimen that is diffracted light or direct light coming from the specimen is directed at infinity by the objective. It is not focused actually rather, it is directed by the objective at the infinity and finally, here the objective is not focusing on light rather the tube length is focusing.

So, when it is directed to infinity it falls onto the tube lens and finally, tube lens focuses light on to the intermediate image plane. And so in essentially when you have to determine the magnification of the objective here, you have to take two things; one is reference focal length, which is from the tube lens till the intermediate image plane. And then you have to consider the focal length of the objective that is real focal length where you are focusing the object. So, magnification of the objective is given by reference focal length divided by focal length of the objective. So, that is how you will be determining here and finally, then as you have seen.

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Let us see this in the ray diagram as we it will be much simpler here, you can see like, we have shown you in the finite corrected optics. Here the objective lens is there, there is a tube length and there is the space between the objective, and tube lens is called infinity focal space or infinity space. Now, this space is utilized for insertion of different auxiliary components, which you can insert in prism or polarizer or analyzer whatever you would like to for your particular application, and that has made lot of advancement and a lot of variations and different types of microscopes. And you can see the rays here, which are coming a diffracted or direct rays, which are coming from the object are focused at infinity that is parallel rays are almost coming out on here.

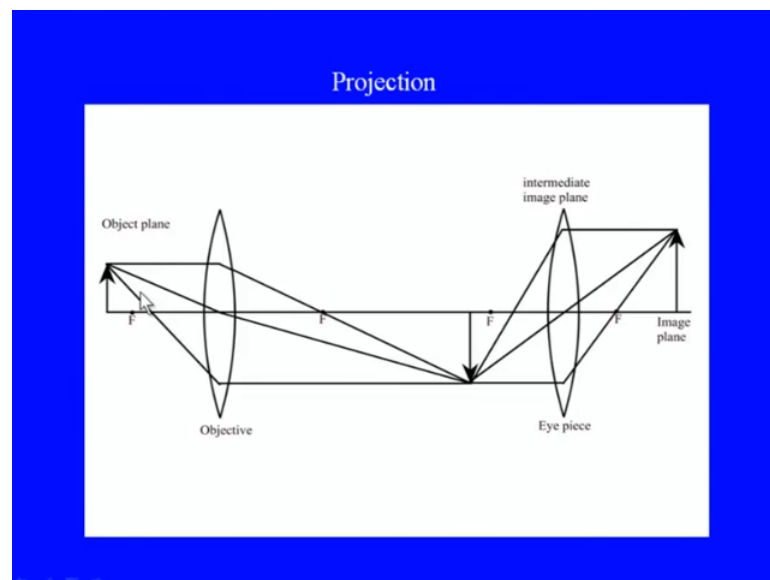
These parallel rays are then or infinity focused rays are then focused finally, focused on intermediate image plane by tube lens. And like in other systems virtual image is formed here, which is erect and which is around 25 to 50 millimeter or 25 centimeter as you say. And then finally, eye lens will form a magnified image, which is inverted on the retina, but upright on the perceived upright by us by the viewer actually.

So, you can see that it is completely different and this like here, there is no image formation most of abrasions could be corrected in objective lens or they could be corrected in tube lens. Most of the time objective lens is corrected for abrasions and it could be or it could be a tube lens. So, infinity corrected, now here one has to understand

it is not like its infinity space or something infinity corrected only means, that rays are focused at infinity.

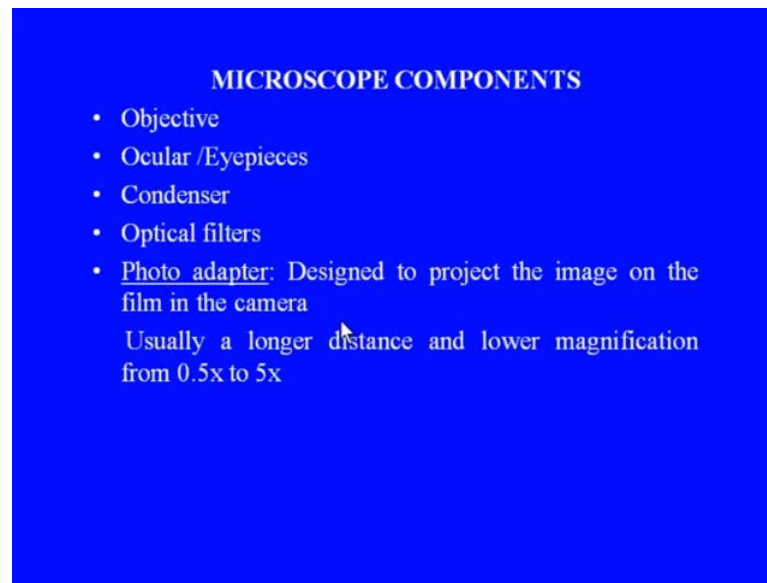
And finally, they are brought to intermediate image plane by tube lens. So, modern microscopes most of them contains infinity corrected objects as we said. Now, supposing there is no direct viewing like you are not observing directly the image, but you would like to record the image for permanent storage or viewing it later or analyzing it later then little differences has to be put in here.

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You can see here, that the main difference is that earlier what we were doing is? Intermediate image plane was within the focal length of the eye piece. That is your image formation that is at intermediate image plane was smaller than one focal length of eye piece, but now here it is little farther than eye piece focal length. So, that now virtual image will not be formed at the same side of the eye piece length rather, it will be real inverted image magnified image will be formed on other side of the eye piece lens, which could be collected on a screen, or could be recorded on a video camera for further applications. So, projections can also be done like, in case of trinocular port and you are putting in camera in there you would like to record the image rather than viewing it directly. So, this was little bit about different types like, we have seen infinity corrected finite optics system and different parts.

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Let us discuss these microscope components in detail and three main components, which we are going to discuss in here. The most important is objective lens like, I told you a simple lens is made up of a single lens, which is called objective lens and it is called objective because it close to objective. And second is ocular lens or eye piece lens, which is closer to eyes where you are viewing the object.

Now, condenser lens is the part of illumination systems and it focuses a fine beam of light on to the specimen in a very small cone, and you will see what kinds of illumination system are available. If you recall the history we have told you are Kohler illumination, so there is Kohler illumination and there is critical illumination. We will discuss that then there could be optical filters there could be diaphragms, or as we say apertures there could be photo adapters, which are there, which are utilized for filming or the in camera.

Now, here we are going to discuss three of them that are objective ocular and condenser lens. Now, most of the objectives in the modern microscopes are standardized with the respect to numerical apertures or magnification. Now, remember anti magnification is nearly allowed here, that is magnification is never empty because you increase the numerical aperture as the magnification needs to be increased.

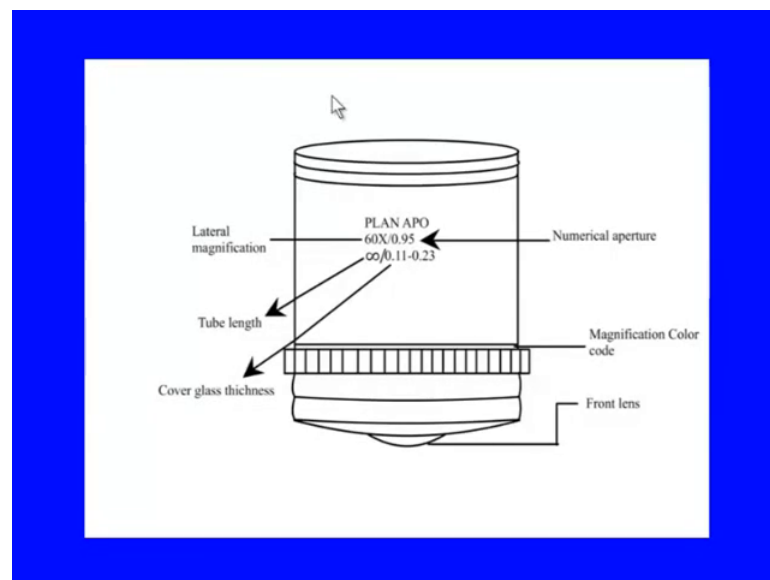
Numerical aperture can be increased by decreasing the focal length, if you could recall we have discussed this where numerical aperture is the light gathering capacity. And it could be increased by decreased wave length you can increase it by not decreasing

wavelength that is the separate thing, but you can increase it by decreasing the focal lens you can bring the lens closer to the specimen, so that more light could be collected at higher angle.

Now, also lens diameter decreases by increasing magnification, so this also could be done then there are like. We have discussed earlier it could be oil immersion lenses or lens working in the air, or it could be water or glycerin all of the have different refractive indexes and accordingly they will give you the resolution. So, high magnification in oil immersion is achieved by placing the object almost within the lens very close to the lens.

Since, it has a very high numerical aperture because of this in the oil immersion lenses. There are residual chromatic aberrations and those could be corrected by compensating eye pieces called Apochromatic lenses. Also objective lenses are corrected for spherical and other aberrations there are flat field objectives, which are, are plane objectives, which are corrected for different aberrations at flat field objectives are corrected for curvature of field. It is required for the microscope when you are doing photo micrography and you are correcting the larger area.

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This figure shows a typical objective lens as we can see here. The objective lens, the size of the objective lenses is bigger than a single lens because this consists of a series of glass lenses, and which are there to correct all spherical. And other aberrations there are few things written in here, which we should take a note and of every objective will be

inscribed in that. One is plane and APO, which is for corrected spherical and chromatic aberrations. Then magnification 60 x here means, that the image could be magnified 60 times that is what magnification shows here? It could be 40 x, it could be 5 x, 10 x the numerical aperture if you see that is the 0.95. Now, remember as magnification increases numerical aperture also has to increase to avoid the empty magnification, so it could be right from very small to very high.

Now, 0.95 is almost highest in the case of lenses, which are being used in the air this here tube length. If you see the infinity sign shows that it is infinity corrected octaves this microscope contains or this objective is infinity corrected. Another is this is power glass thickness given many objectives will have a color code for different kinds of magnification.

And there is a front lens, which is very close to the specimen and the working distance will be calculated from the front lens to the specimen where it is placed on the specimen stage. So, objective lenses, now if you compare certain properties of objective lenses like, we are talking about when you increase the numerical aperture then the it will reduce the focal length. So, there are few things, which we need to relate here.

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**PROPERTIES OF OBJECTIVES LENS**

Magnification	NA	Focal Length (mm)	Working distance (mm) <sup>1</sup>	Diameter of field (mm)
10	0.25	16	5.5	2.0
20	0.54	8	1.4	1.0
40	0.65	4	0.60	0.5
40	0.95	4	0.25	0.2
95 <sup>2</sup>	1.32	2	0.10	0.05

<sup>1</sup>Distance from front surface to lens of the sample.  
<sup>2</sup>Oil immersion lens.

For example, when you increase the magnification as shown in this table from 10 to 95 x. You can clearly see that the numerical aperture also increases likewise and, but focal length decreases that is you are bringing the objective lens closer to the specimen. So,

you are decreasing the focal length also the working distance decreases. The working distance as I said, the difference between the front length and the specimen and this will decrease as you increase the magnification and as you increase the numerical aperture. Diameter of the field that is the area, which you can view, will also decrease because farther, you are from the specimen more are could be seen as you go closer to specimen less area you will see we will discuss the diameter of field later on.

So, this is about the objective lens, which is like as I said, very much standardized for magnification and resolution. Let us, now move onto the next important part of the microscope, compound microscope eye pieces or ocular lenses. Now, ocular lenses are also called projecting lens, or projector lenses because what happens is they are projecting the image, which is formed already by the objective lens. Remember objective is the main lens in the microscope, and the resolving power of microscope is solely depending on the objective, because objective collects the rays, diffracted rays or most of other information directly from the specimen. And the eye pieces will collect the information from the image formed by the objective.

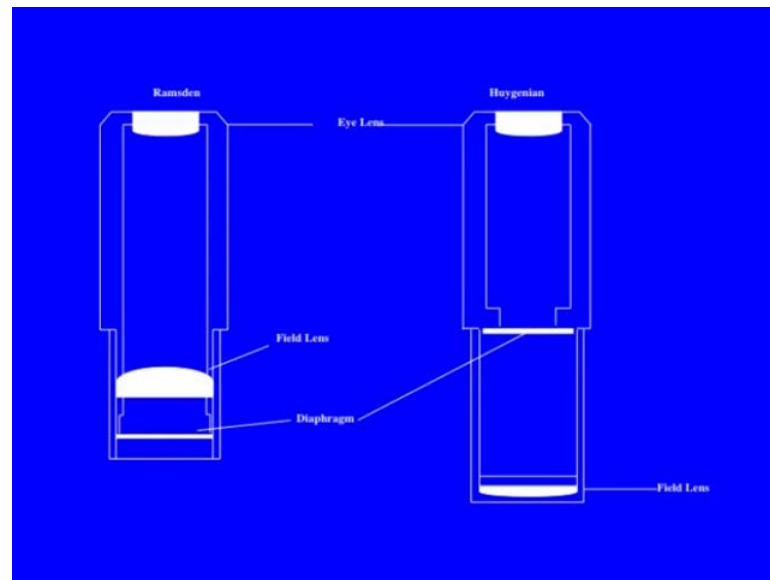
So, there cannot be additional information, which eye piece will collect only thing it can do is it can magnify the image to a certain label, so that more information can be viewed or observed. It is always better to have a higher magnification objective than eye piece. For example, 40 x objectives with a 5 x eye piece will give you much greater resolution than a 10 x objective and 20 x eye pieces.

So, one has to remember that there are different types of eye pieces there are many different kinds of eye pieces and we are not going to go in all of them. But some of the names here I have given, which are used frequently like Ramsden, Huygenlan and Caliner and compensating eye pieces. Compensating we have discussed like, in oil immersion lenses for correcting the chromatic abrasions residual chromatic abrasions, the compensating eye pieces are utilized.

Now, Ramsden and Huygenlan eye pieces are widely used, but there are many variations that have come up these two consist of two lenses at opposite end of the tube, the tube which is connected to the one end of the mechanical tube length tube. So, what you have is? Two lenses, one is closer to the eye, which is called eye lens and another is at the other end, which is called field length.

So, these two pairs of lenses are available with as a diaphragm either in between the two lenses or at one end of the lens as we will see in this figure. Now, if you consider parallel lens it is modified version of Ramsden with a doublet of eye lens, and it features a higher field of view for eyes or you can say higher eye point and a larger field of view.

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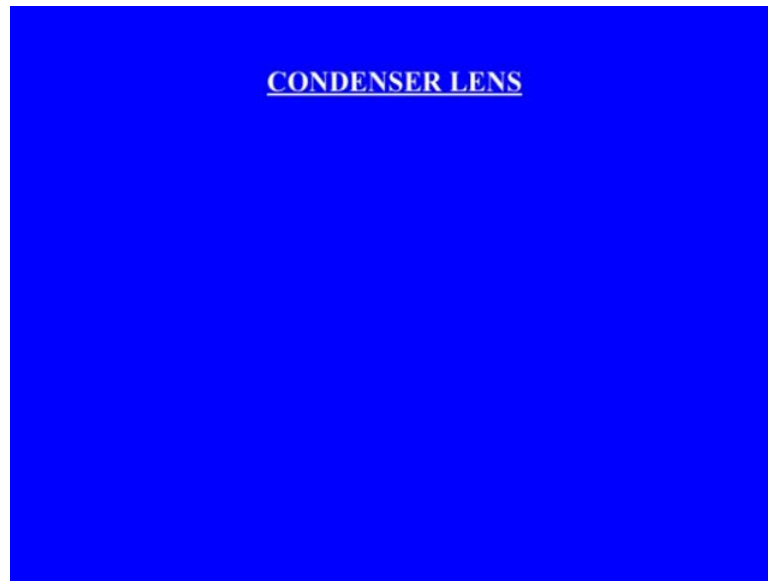


Now, these are schematic of the two kinds of lenses, which we were talking about this is huygenial lens. You can see huygenial lens there is eye lens here, there is field lens at other end of the tube and in between this there is a circular aperture opening, which will determine the field of view it is called diaphragm. Now, if you could see that the convex part, the Plano convex lenses and the convex part of the lens focuses on to the specimen side these are also called this is also called negative eye pieces.

Now, in comparison Ramsden eye pieces are also Plano convex lens, but if you could see that the convex part of the field lens faces. The eye lens rather than towards specimen also the diaphragm is not situated between the two lenses rather, the diaphragm is situated below the field lens. And this you can say you can mount adaptable for mounting radicals Ramsden are adaptable for mounting radicals, and their focal length is kind of at the diaphragm here. So, these are called positive lenses and they are used in many different applications. Huygenial lens are also used for many teaching ends and research labs there could be many other eye pieces, which we are not going into.



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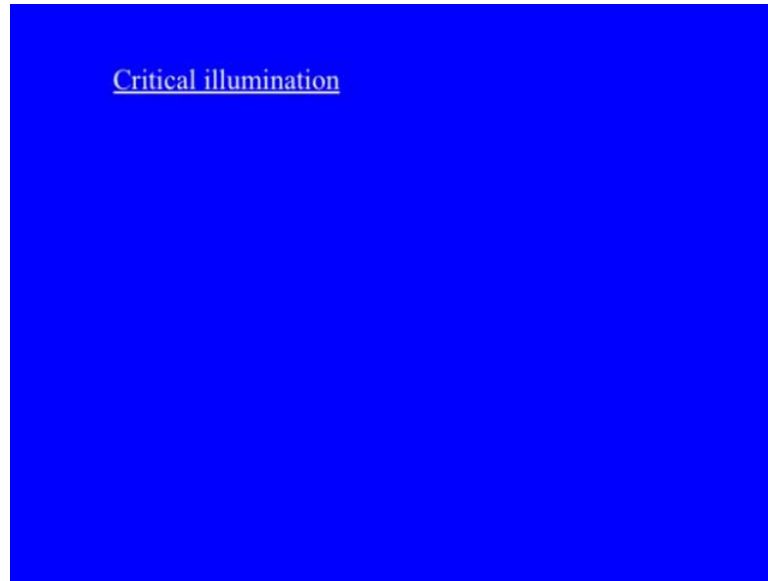


The third component is the condenser lens. Now, condenser lens are a very important part of illumination system. Now, there are few conditions, which need to be fulfilled for like, object to be illuminated in an ordinary microscope. What are those conditions? One is that incident light must fill the numerical aperture to ensure the resolution of objective that is the best resolution of objective that, so first thing is that the numerical aperture should be completely filled to get complete resolution of objective. Second is the illumination should be across the specimen that is it should not be like, one part is illuminated more than another. So, it should be uniform illumination across the specimen has to be there and one has to eliminate all stray light or noise.

Now, condenser lens play an important role to fulfill these criteria. So, what has to be done? One is that a small dimension of light is focused down to a very small area by using multiple lenses in a single lens unit called condenser. Now, there could be different types of condenser four types of condenser lenses one is able, aplanatic, achromatic and aplanatic and achromatic lens.

Now, able lens is not corrected for any abrasions there were many simple lens are used in their aplanatic lens are those, which are corrected for spherical abrasions and achromatic lenses are corrected for chromatic abrasions, aplanatic and achromatic lens are corrected for both abrasions. So, here these are four types as you have more features they are more expensive also.

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Now, condenser can provide two kinds of illumination; one is called critical illumination another is called Kohler illumination.

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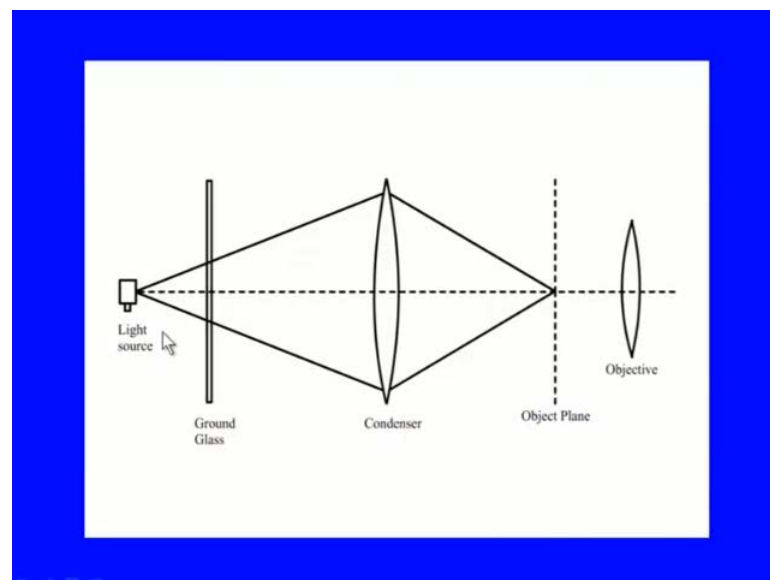


Now, object is illuminated in cone lets discuss critical illumination object will be illuminated by a cone of light from a focused light source. Now, the position of condenser with respect to the object is adjusted, so that to ensure that the spot of illuminated of that object is as small as possible it is a cone. You can say a small cone of light is illuminating the object also, it should be as bright as possible it should be as

intense as possible. And most of the times a diffused screen is portrayed before the light source like I told you, so that the light source is not filled here, not imaged here.

Now, numerical aperture needs to be at least as great as the objective in order to fill the object aperture that is a very important part. If you cannot fill the objective aperture you cannot get the required resolution of the or the full resolution of the objective lens. So, numerical aperture of condenser lens should be greater than numerical aperture of objective.

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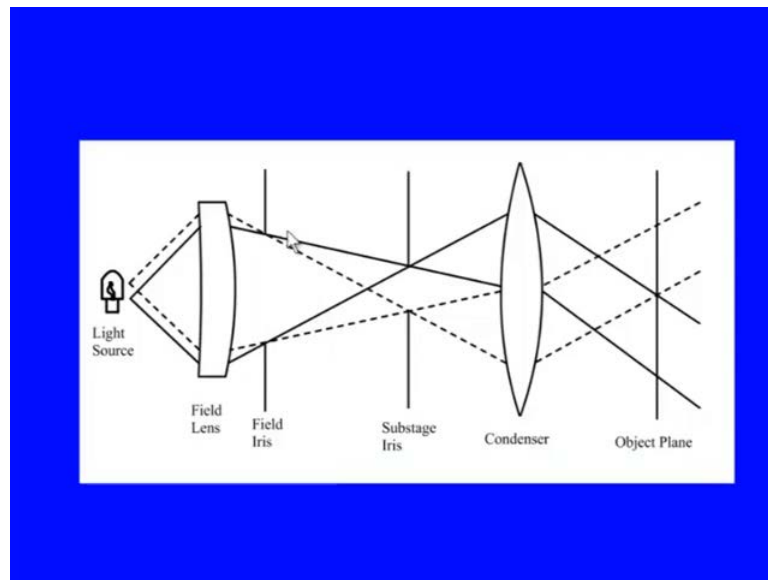


Now, this a very simple schematic diagram to show the critical illumination it is simple light source illuminating will be illuminating the object. And condenser will focus the light on a very small area stray light has to be avoided through different apertures or diaphragms, which are placed at different places at different sites. A very small cone of light will be illuminated the object on the object plane. And finally, the diffracted and non diffracted ((Refer Time: 34.40)) the objective. So, critical illumination is a simple illumination, but not it has been replaced by Kohler illumination, which is much more intense and precisely controlled as compared to the critical illumination.

Now, in this one there is an extra or excessively lens or field lens to be displayed between the light source and condenser. When light rays can leave the condenser it leaves as the parallel beam of light many parallel beams of light are called the pencils of light. So, you can say there are many small cone of light, which are falling on the

specimen at different angles actually. So, all light passes through the object at various angles, and the angles will increase with the distance of the source point from the optical axis. So, access is here, illuminated by sets of pencils that makes up the for the cone of light, which was for critical illumination.

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Now, condenser can be adjusted and it made sure that the stray light is avoided. If you see in this figure, this figure shows you a very simple schematic of how the Kohler illumination is achieved. You can see there is a field lens here and this field lens and there are field irises or sub stage iris at different places, which will not allow the stray light to pass on. And finally, the object is illuminated rather than one cone of light many pencils of light are illuminating the object uniformly. And this is much more precisely controlled and you can get intense uniform beam to illuminate the object and, which is used in most of the microscopes.

So, this was about critical and Kohler illumination by the condenser we have discussed the three kinds of lens system, which are important in here objective, eye piece and condenser. Now, there are few terms, which need to be discussed in here, before we like move onto the particular microscopic techniques. One, which you remember the objective we were trying to discuss like field of few other things before that there two terms depth of field and depth of focus.

Now, depth of field is thickness of a specimen that can acceptably be sharp at a given focus label, so that depth of field if it is a particular thickness of the specimen can be at focus then it is depth of field. So, once you go more than that then focus is lost. Another term is depth of focus, depth of focus refers to the plane over, which the image plane can be moved, while an acceptable amount of sharpness is maintained. So, that is depth of focus field of view, which we were discussing this earlier.

Now, field of view is another important thing that how much area of specimen you are viewing. So, diameter of field in optical microscope is expressed by field of view number or simply field number. Now, this is the diameter of view of field in millimeter majored in the intermediate image plane in most cases. Now, the eye piece field diaphragm opening diameter determines the view field size.

If you could remember in the Ramsden and Huygenial we have shown that there is a diaphragm that is circular aperture and that will determine the field of view. And field size in specimen plane is then defined as the field number divided by magnification of objective. So, when we would say field view that was in intermediate image plane, the field size is at the object or specimen label. The field size is given by field number and divided by the magnification actually that is objective magnification. So, you can determine the field size in the specimen plane by these simple formulae.

Another important part like, we have discussed like resolution like magnification, but another important part of viewing an object or specimen is the visibility. Now, unless different components or different parts are visible that is they could be differentiated like, different points in the object can be differentiated from each other. And differentiated by the background you will not be able to see.

Lot of biological specimen are transparent and many times though they could be observed, but they are very hard to be visible in the image because the intensity differences between them between the two objects in the specimen, or between the object and the background is too low, so they could not be seen. Now, here comes the term contrast, so there has to be contrast for visibility. What is contrast? Contrast can be defined as the difference in light intensity between the object image object or image and the background.

Now, here the difference between color and brightness of an object from other objects in vicinity and background creates contrast and allows the details of an object to be visible. For human eye minimum contrast value is 2 percent that is 0.02, which is required to be distinguished between an object and its background. And it could be given by simple formula that is percent contrast could be calculated by  $\frac{I_S - I_B}{I_B} \times 100$  that is the background I S. The specimen intensity minus I B, which is background intensity into 100 divided by the I B, which is the background intensity. Like, I said most biological specimen are transparent and are not visible because of they lack contrast and there has to be created contrast by different techniques.

One is staining the object with particular dyes ((Refer Time: 41.08)) and those dyes can absorb light and can give intensity differences, but there are other methods also, which are also utilized for creating contrast like. We were going to discuss in coming classes, in the in the coming lectures how the contrast could be created without staining the objects. Now, when you stain the object or the specimen what you do is? To certain extent you cannot observe light cells, or the cells as it is there will be some differences in observing the light cells and the stained cells. Other than stating there could be different optical phenomenon could be used to create contrast.

For example, we will be discussing dark field microscopy, phase contrast microscopy, fluorescence microscopy, polarization microscopy for interference microscopy to see how contrast is developed in these different techniques. And then also we will be discussing how specimen will be stained? How this sample is prepared for the observation under a microscope? So, in the next lecture, we will be discussing the specific techniques and we will start with dark field and phase contrast microscopy.

Thank you very much.