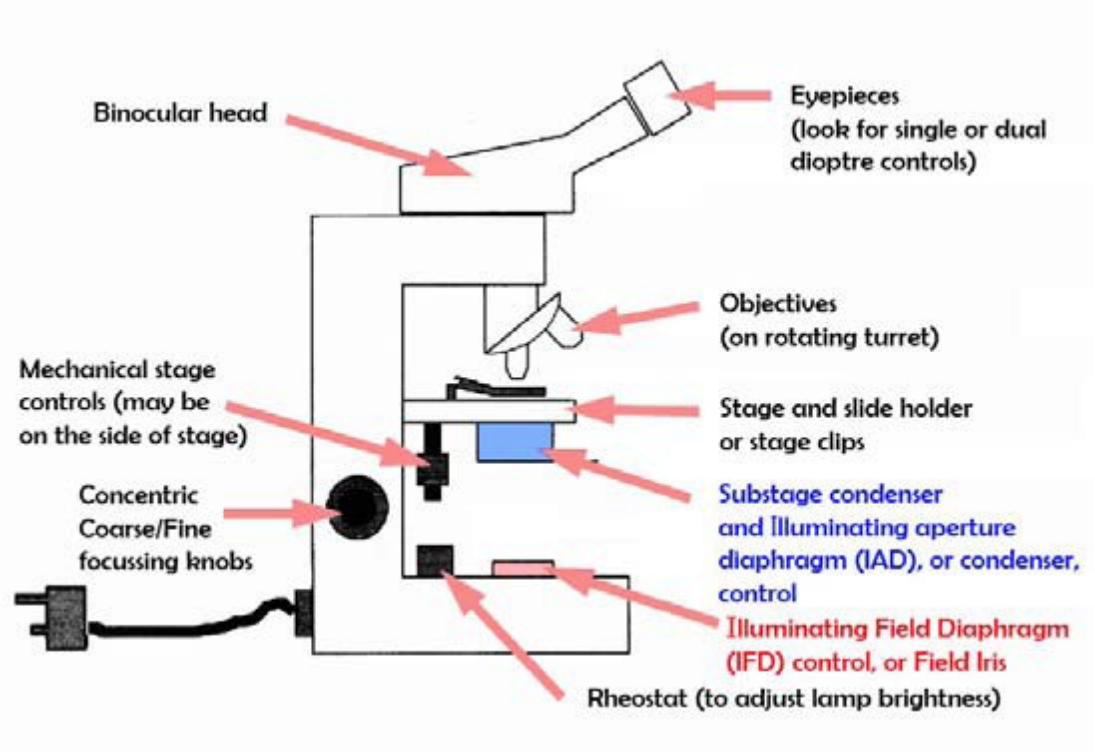
HOW TO SET UP A MICROSCOPE FOR TRANSMITTED LIGHT ILLUMINATION

Köhler Illumination

1. Turn on the light source and swing in a 10x objective. This is of sufficiently low magnification not to hit the slide while initial adjustments are being made.

2. Place a well-stained slide on the stage, checking that the coverslip is uppermost. Now, whilst looking into the eyepiece(s), focus the image with the coarse focus control. Adjust as necessary with the fine focus control. This sets the specimen in correct relation to the objective. If the light is too bright, reduce it with the rheostat on the light source.



3. Decrease the size of the illuminating field diaphragm (IFD), sometimes called the field iris, located nearest the light source so that you can see its edges. Focus the condenser slowly with the condenser focusing knobs usually situated under the stage until the image of this diaphragm is sharply in focus at the specimen plane, superimposed upon the image of the object.

4. When the edge of the field diaphragm silhouette is sharply defined, centre it with the two knobs (usually knurled knobs) coming out diagonally from the condenser. Close down the field diaphragm most or all the way to get it centred properly. When it is centred, open the field diaphragm until its edge is just outside the field of view. Do not open it too much, otherwise stray light will reduce contrast in the image.

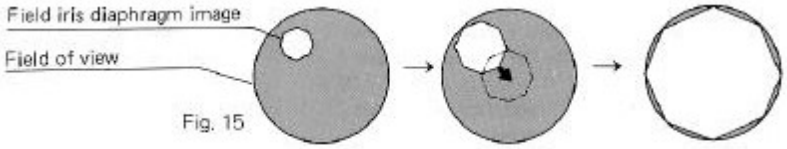


Fig. 15

5. The correct height of the condenser has now been set. The illuminating aperture(IAD) can now be adjusted so that the aperture of the variable cone of light supplied by the condenser can be correctly matched to the (generally) fixed numerical aperture (NA) of the objective. In theory, the aperture of the condenser should equal that of the objective. However, in this case stray light refracted from the extreme edges of the objective lens elements would cause an appreciable loss in contrast. It is worse, however, to close down the aperture diaphragm too far: this will cause serious degradation in image quality, and loss of resolving power. This diaphragm is not be used to control brightness in the image; rather, use the rheostat control on the lamp transformer, or (where the intensity of the lamp must remain constant, as for colour photomicrography for example) use neutral density filters. Closing down the aperture diaphragm from its optimum position will increase contrast at the expense of severe loss of resolving power. Decreasing the aperture of the condenser will also increase its depth of field, and bring into focus dust and other contamination from the surfaces of the specimen preparation normally invisible in the properly adjusted microscope.

6. Your specimen should be properly illuminated and should give you a great image. If it does not, check to make sure your lenses and other optical components are clean. Then recheck to see that you have followed each step properly.

Adjusting the Oculars

You may not need your eyeglasses when using a microscope, unless they correct for astigmatism. Using a single ocular, the focus control alone can bring an image into sharp focus. If you have a binocular microscope, the eyepieces should be adjusted to compensate for eye differences.

Anyone who has used binoculars should find it easy to adjust the oculars on a binocular microscope. Before even focusing on a specimen, you should be able to adjust for eye separation so you will see a single field of view. When the oculars are separated to match your eyes, you should be able to look into them with both eyes relaxed, just as if you are looking across a room. If you have trouble with binocular vision, you could be among the minority of users with eyes set close together, making such viewing difficult. It is more likely, though, that the individual oculars are simply out of adjustment, which prevents you from bringing the image into focus for both eyes at the same time.

Your microscope may be equipped with one fixed and one adjustable eyepiece, or with both eyepieces adjustable. Either way, the first step is to place each adjustable eyepiece in the center of its range of travel, giving you the most latitude for adjustment either way. The next step is to obtain an image at high enough magnification so that you can see fine details. Step three is to observe with the fixed eyepiece only (or one of the two adjustable eyepieces) with the appropriate eye, and focus the microscope on the image. Recalling one or two specific

details from the image, observe with the other eye only, and this time, adjust only the eyepiece until the details come into focus. From this point on, when you focus the microscope, you should be able to look comfortably using both eyes.

If you had trouble seeing a single image when adjusting for eye separation, it may be worth trying again once the oculars are adjusted to match your eyes.

1. Set microscope up carefully using a fine-detailed specimen, and change to the highest dry objective.
2. Set intraocular distance on binocular head (if fitted) for comfortable use.
3. Focus precisely using the fine focus adjustment.
4. Change to lowest magnification objective but **do not** readjust microscope focus controls.
5. Focus image for each eye by using the individual adjustment for each eyepiece.

6. Repeat steps 1. to 5., and the microscope should then maintain focus throughout its magnification range