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Köhler Illumination: A simple interpretation

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The problem

Even for visual use, but especially for photomicrography, it is desirable that a microscope object should be illuminated uniformly across the field of view. One of the simplest ways of doing this is to throw on to the object an image of another area which is uniformly illuminated. In Köhler's time this was a large white cloud in the sky, or by night the flame of an oil-lamp, imaged on to the specimen by means of a lens which we call the condenser. In this connection the condenser can be considered to operate in just the same way as a camera lens, producing an image of a relatively distant object in its second focal plane. This kind of illumination is known as 'source-focused', because the source is focused on to the object; in older texts it is also described as 'critical' or 'Nelsonian' illumination.

Source-focused illumination

Source-focused illumination can be entirely satisfactory, especially for use by the 'amateur' or in a teaching laboratory lacking more complex equipment. It requires a microscope, usually fitted with a mirror, and a light source which is sufficiently large, bright and uniform. Several types of electric lamp are available, which provide a uniformly-illuminated area of several centimetres diameter. These include the standard fully-diffusing opal bulb designed for photographic enlargers, and some of the special 'decorative' bulbs. Experiments with the use of electronic flash for photomicrography can also make use of source-focused illumination: a sheet of white paper illuminated by the flashgun can be imaged (via a mirror) by the condenser on to the object.

Köhler's innovation

Köhler's initial difficulties were caused especially by the irregularities of the light sources available to him, for example the net-like weave of the gas mantle, which if imaged on to the object would provide an objectionable background especially in a photomicrograph. He considered the use of a diffuser to be unsatisfactory, since it would waste too much light.

Köhler decided, in contrast to source-focused illumination, to shift the image of his irregular light source as far away as possible from the object, namely to infinity. Rather than place his gas-lamp actually at infinity, from which distance it would appear very small, he explored an optical equivalent of this, using the condenser lens. If a source is placed in the first focal plane of the condenser lens (where we are accustomed to finding the condenser's iris diaphragm), from the object's point of view light arrives in the form of a parallel beam, as if from infinity. Microscopes have been made according to this principle, using a small electric lamp in the first focal plane of the condenser, but clearly the type and size of light-source are restricted, and heating of the object (and the rest of the microscope), particularly with a gas lamp, would be a problem. Köhler introduced an extra lens, which we now call the lamp collector lens, and moved the light-source back, so that an *image* of the source was projected into the first focal plane of the condenser. This situation is essentially similar to having the light source itself in this position, and makes it possible to include a diaphragm for regulating illuminating aperture here.

The essential features of Köhler illumination

Perhaps the most 'special' feature of Köhler illumination is the way in which the lamp-collector lens functions, producing a *uniform* disc of light from an *irregular* light-source. How this works can easily be explored using a lens of 30 or 40mm diameter and of 50 to 100mm focal length (a lens from a single lens reflex camera serves well) to represent the lamp collector, together with a small electric lamp (I use a 'Maglite' torch). Hold the lens almost at arm's length with its larger-diameter element (if any) towards the eye, and hold the torch with its lamp close behind the lens; slowly move the lamp backwards, increasing its distance from the lens. When the lamp is too close, an upright image of the lamp will be seen within the lens, and when it is too far this image becomes inverted; but at a special position which lies in between, the lens will appear uniformly filled with light of possibly uncomfortably high intensity. Perform the experiment in front of a mirror and note that, when the lamp filament and the lens are in this special relationship, an image of the filament falls on to the eye (displace it a little on to your face or forehead to see it). This demonstrates the secret of Köhler illumination: how a lamp-collector lens can provide a uniform and intense patch of light of considerable area, even when using a small and irregular light source. When imaged into the object plane of a microscope, this patch of light serves as an ideal secondary source of illumination. In performing this experiment you will have noted that precise positioning of the lamp and the lens is critical; the optical axes of the lens and of the eye must coincide, and the lamp must lie on this common axis. Repeat the experiment holding a piece of tracing paper close to the lamp; adjustment now needs to be less precise - one reason why many manufacturers fit a diffuser between lamp and collector lens.

A step-by-step analysis of Köhler illumination

A microscope correctly set up for Köhler illumination has several important features which can be appreciated from the accompanying diagrams, which show the two sets of conjugate planes in a transmitted-light microscope (Fig 1).

a. The light source, nowadays usually the filament of a tungsten halogen lamp, is imaged by the lamp collector into the first focal plane of the condenser (Fig. 1 a). Since the filament is thus deliberately placed in the *aperture* set of planes, its irregularities cannot disturb the illumination of the objector its images, which lie in the field set of planes.
b. The lamp collector lens appears uniformly illuminated when in this configuration.
c. An iris diaphragm placed just after the lamp collector is imaged on to the object with the condenser lens (Fig. 1 b). Since this diaphragm limits the extent of the field of view which is illuminated, it is known as the *illuminated field diaphragm.*
d. This image of the lamp collector, bounded by the illuminated field diaphragm, provides a flawless field of uniform illumination.
e. The illuminated field diaphragm should be closed to confine the area illuminated to that under observation by a particular objective lens. This in turn restricts the illuminated area of the primary image to the diameter of the field diaphragm of the eyepiece, and prevents light from falling on the internal walls of the microscope tube, and possibly giving rise to disturbing reflections. Note that the illuminated field diaphragm acts as the control for *area* of illumination.
f. An iris diaphragm is located in the first focal plane of the condenser, where the image of the lamp filament falls. All rays of light entering the condenser lens from any individual point in this plane pass parallel through the object, as if from infinity. These rays are brought together in the back focal plane of the objective, where a (usually fixed) diaphragm is located. This *aperture diaphragm of the objective* limits the effective numerical aperture of the objective.
   A further image of the filament is formed here, now bounded by the condenser diaphragm which, because it limits the extent to which the aperture of the objective is filled with light, the 'illuminating aperture', is known as the *illuminating aperture diaphragm.*

g. The illuminating aperture diaphragm should be closed so that the illuminating aperture (the included angle of the cone of rays entering the object) is just a little smaller than the imaging aperture (the included angle of the rays collected by the objective from the object, normally expressed as the numerical aperture of the objective). Amongst other things, this will avoid the effects of stray light scattered from the mountings and the edges of components of the objective lens. Note that the illuminating aperture diaphragm acts as the control for *angle* of illumination.

In the accompanying diagrams, only on-axis rays arising from the filament (Fig. 1 a) or passing through the object (Fig. 1 b) have been shown. Other similar diagrams could be drawn for non-axial rays, which would come to a focus in the same planes as the axial rays.

The diagrams illustrate another elegant feature of Köhler illumination. Fig. 1 (a) shows that rays arising from *each individual point* on the filament pass through all parts of the object under observation, and Fig. 1 (b) shows that *each individual point on the* object receives light from all parts of the filament admitted by the condenser aperture.

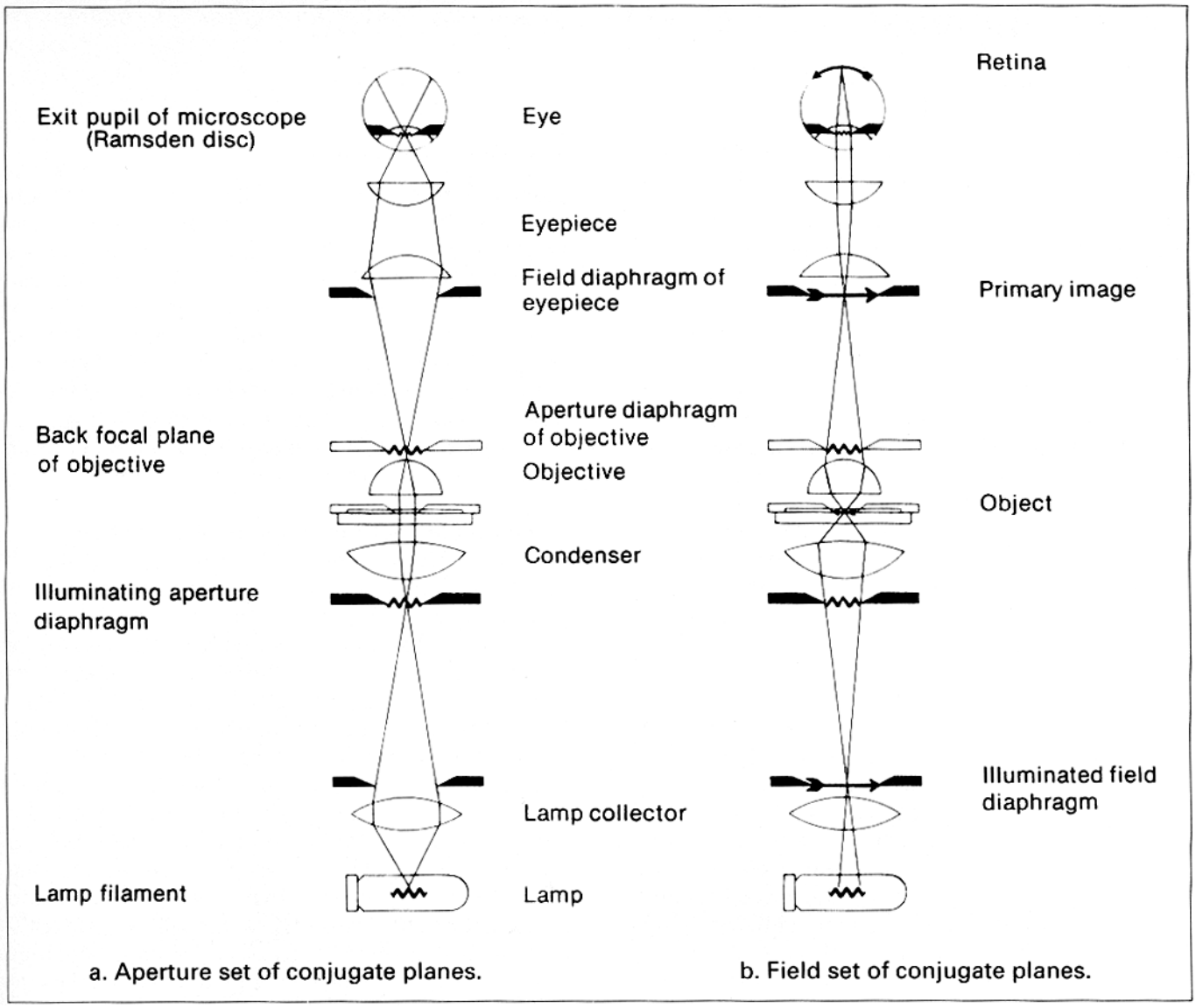


Fig. 1. Ray paths of the transmitted-light microscope showing (a) the *aperture* set and (b) the *field* set of conjugate planes. From the RMS Dictionary of Light Microscopy.

Observing the differing effects of the diaphragms

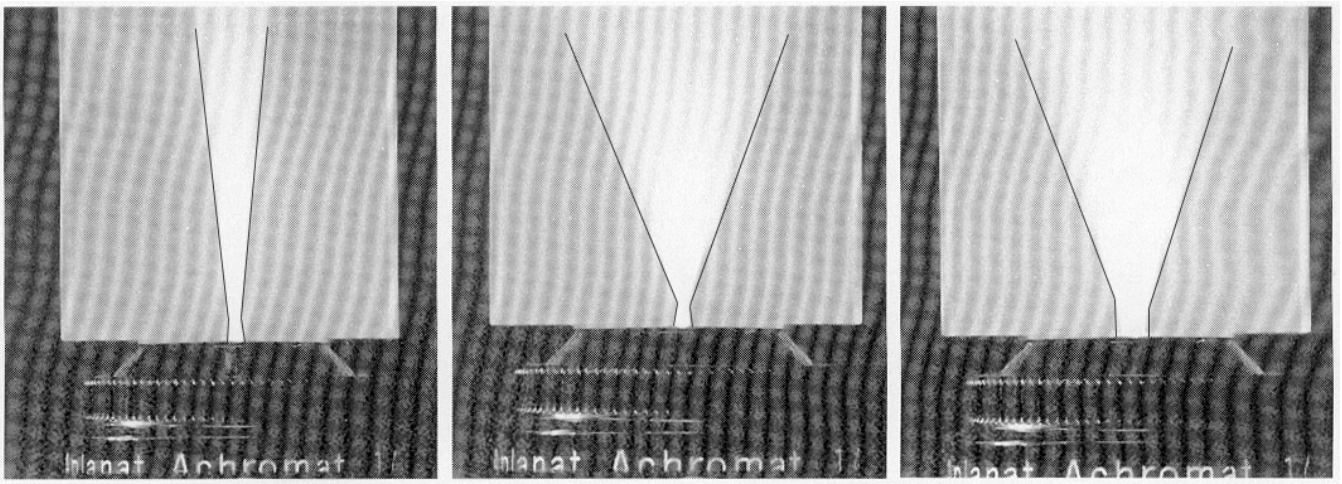
It is very instructive to study the separate effects of the microscope diaphragms on *area and angle of* illumination. Some means must be devised to observe the passage of light. This is most elegantly done using a block of slightly turbid glass; on our teaching courses we use pieces of fluorescent red perspex with polished edges. An adequate substitute is a *very dilute* solution of milk in a transparent container, for example a coverglass box. Raise the condenser to the top of its travel. Place a drop of immersion oil on the condenser's top lens, and lower the turbid block or its substitute on to the microscope stage. The use of immersion oil is not essential, but it provides good optical contact and makes the observations more dramatic. Open all diaphragms, switch on the lamp, and observe the rays as they pass between condenser and objective.

Open and close the illuminating aperture (condenser) diaphragm: note how this diaphragm controls the included *angle* of the rays. This is shown in Fig. 2 (a, b).

Leave the illuminating aperture diaphragm in the centre of its range and close the illuminated field diaphragm; note that the *angle* of illumination remains unchanged, but the *area* illuminated decreases (this is represented by the diameter of the 'neck' at the apex of the inverted cone of rays leaving the object plane; see Fig. 2b and c).

Set the illuminated field diaphragm in the centre of its range and adjust the illuminating aperture diaphragm again; this diaphragm alters *angle* of illumination while the area remains unchanged.

It should be clear from these observations why the illuminated field diaphragm must be set according to the *magnification,* because this determines *area* observed, and the illuminating aperture diaphragm according to *numerical aperture* of the objective in use.



(a) (b) (c)

Fig. 2. Rays leaving the condenser lens, made visible by their passage through a turbid glass block, to show the effects of varying the illuminating aperture diaphragm (a and b - note change in included *angle* of cone of rays) and the illuminated field diaphragm (b and c - note change in *area* illuminated, at apex of inverted cone of rays). (a) illuminating aperture 0.25; (b) illuminating aperture 0.65, illuminated field diaphragm closed; (c) illuminating aperture 0.65, illuminated field diaphragm open. Lines have been overlaid onto the original figure to show clearly the limits of the cones of light.

Köhler illumination in the modern microscope

Most advanced modern microscopes from the major manufacturers employ a system of Köhler illumination essentially as described here, some with the addition of extra lenses to extend the area illuminated. There have been several variations on the basic theme. Leitz (and now Leica) have made use of two interesting developments. A condenser containing both the field and the aperture diaphragm was fitted to the Ortholux from 1940 until the 1960s. Designed by Max Berek (1886 -1949), this condenser is effectively a complete Köhler optical system, lamp collector and condenser, axially compressed to a length of about 50mm. The second variation is seen in the new DMR range of microscopes - the condenser in this case has no diaphragms. The illuminating aperture diaphragm is positioned within the base of the microscope, in a plane optically equivalent to that normally occupied by the filament of the lamp; the filament is imaged into this plane by an extra lens mounted in a lamphouse at the rear of the microscope. Both the filament and the illuminating aperture diaphragm are imaged into the lower focal plane of the condenser. This system has the advantage that the microscope can be operated without raising a hand to the condenser, and should also offer the designer greater freedom: with some types of condenser the lower focal plane is not accessible to accommodate the iris since it lies within the lower lens element.

This system in which the illuminating aperture diaphragm is remote from the condenser itself is, of course, the one which has been adopted for many years in the bright-field epi application of Köhler illumination, where the objective serves also as the condenser, since any diaphragm fitted in that lens to control the illuminating aperture would also restrict the imaging aperture. An epi-illumination system has a further inherent simplification: when the objective is changed, the condenser is also changed - for one whose aperture and field exactly match those of the objective, since they are one and then same lens. This removes the need for the constant readjustments of the two diaphragms, which are necessary with the conventional transmitted-light system where one condenser is generally used to illuminate a wide range of objectives.

It is a pity that the pancratic condenser never really found favour. This is a 'zoom'-type condenser of variable focal length, in which both area of field illuminated and illuminating aperture could be varied together, in an inverse manner, so as to approximate the normal combination of field of view and aperture of objectives. Again, no frequent readjustment of diaphragms was required. The Zeiss KM microscope, a design that was so ahead of its time that it did not remain on the market until this time arrived, made use of a system with a similar effect, as does the Olympus Vanox. Perhaps one day 'real' microscopists will come to accept such devices, which offer correct illumination almost automatically, just as automatic transmission for cars is now almost accepted even by 'real' motorists. Would Köhler have spent his time fitting, removing and stopping-down spare objectives to act as condensers, and cutting discs from black card, had a pancreatic condenser been available to him?