Setting-up the Microscope

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Summary

Procedures for setting up the microscope are given in the form of working instructions (a) for field-focussed illumination, where a large diffuse light source is focussed in the field of view and (b) for aperture-focussed illumination, where a small-filament light source, with lamp condenser, is focussed in the aperture of the substage condenser.

Introduction

THERE ARE many mechanical and optical variations in the design of the light microscope, and instruments range from the simple biological microscope to highly complex multi-purpose units. All, however, have in common an illumination system, with aperture and field diaphragms. Unless the microscopist correctly adjusts and continually controls this illumination, it is not possible to do good work, nor to display the finest detail in the image. Anyone beginning work in a field where the microscope is used should thoroughly master the technique of setting-up. Once the steps involved are understood, the technique of setting-up is easily remembered, and can be rapidly accomplished without reference to instructions.

Modern microscopes may be purchased either with or without built-in illumination. In the latter case, a separate lamp is necessary, either of the 60 - 100 W diffuse kind (Fig. 1) for use with magnifications up to ×400, or of the high-luminance, clear-bulb type (30 - 100 W) with a lamp condenser (Fig. 2), for use at magnifications up to ×1400 and for photomicrography. Both these types of lamp should have one or two filter holders for the insertion of blue (daylight) and other types of filter, and an iris diaphragm for limiting the field of view. With microscopes having integral illumination, the instructions for setting-up given in this paper will normally be applicable, provided that the initial instructions concerning positioning of the lamp and adjustment of the mirror are ignored.

A description of the procedures for the correct setting-up of the microscope is given in this paper for the two main types of illumination. These are (a) Field-focussed illumination (also called critical, source-focussed, diffuse or Nelson illumination) where a large diffuse light source is focussed in the field of view, and (b) Aperture-focussed illumination (also called Köhler illumination), where a small-filament, clear-bulb light source is focussed in the substage condenser aperture. A third type of illumination, useful for looking at interference figures, is large-source Köhler illumination, where a larger diffuse light source is focussed in the substage condenser aperture.

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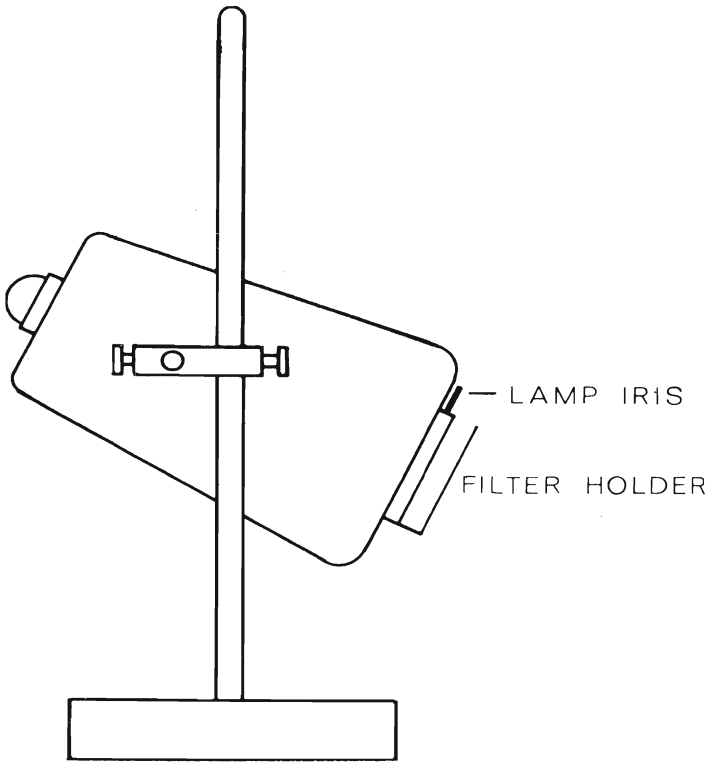

FIG. 1. Diffuse-type microscope lamp.

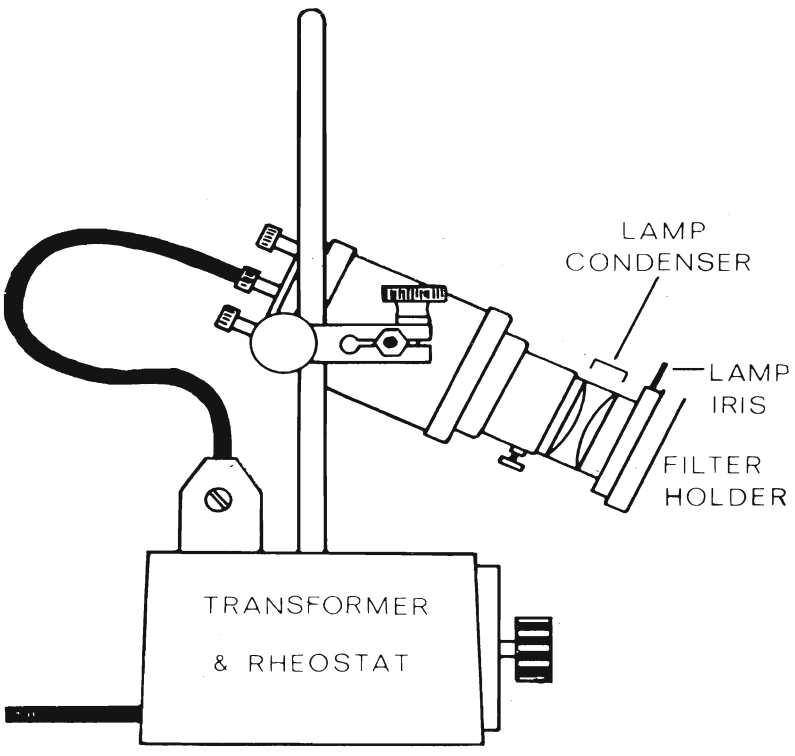

FIG. 2. Condenser-type microscope lamp.

Setting up Field-focussed Illumination

Diffuse illumination is useful for total magnifications up to about ×400 (e.g., 4 mm objective and ×10 eyepiece), and may be provided by daylight, a pearl or opal lamp, or a clear bulb behind a diffuser.

Working instructions for setting-up with a diffuse lamp are given below, the ray diagram being shown in Fig. 3.

(1) Arrange the microscope so that the working condition is comfortable, with the lamp about 20 cm or 8 inches in front of the plane mirror. The microscope may be used either in the vertical position or inclined at an angle of about 30° to the vertical, unless the instrument already has an inclined eyepiece tube; the vertical position is essential for liquid preparations.

(2) Tilt the plane mirror until the light is reflected uniformly up the microscope tube.

(3) Place a specimen slide of reasonable contrast on the stage and, using a 16 mm objective, ×10 eyepiece and 160 mm tubelength, focus on the object. Do this by lowering the objective to near contact with the slide, *while watching from the side,* and by then racking upwards (coarse and fine rotation backwards).

(4) Almost close the lamphouse iris, rack the substage condenser up to near contact with the slide and then rack down until the lamp iris comes into focus. If a pearl lamp is used and the 'grain' is visible in the field of view, lower the substage condenser *slightly* to give an evenly illuminated field. If necessary, centre the lamp iris in the field of view by very slight adjustment of the mirror. Open the lamp iris so as just to fill the field of view. If no lamp iris is available, focus on a pencil point held in the centre of the lamp opening.

(5) Remove the eyepiece, or insert the Bertrand lens in the case of a polarising microscope, so as to inspect the back focal plane of the objective. (An inverted ×10 eyepiece held above the microscope eyepiece will also suffice.) Almost close the substage iris and centre the condenser (or objective) by means of the centring screws, until the image of the substage iris is concentric with the objective aperture. Next, open the substage iris to fill $\frac{2}{3}$ to $\frac{3}{4}$ of the back lens of the objective.

(6) Replace the eyepiece or remove the Bertrand lens and make final adjustments with the fine focus.

(7) If necessary, insert a filter in front of the lamp or in the substage holder to reduce the light intensity, provide contrast, reduce aberrations, increase resolution or to provide a selected wavelength or band of wavelengths. The object is now being viewed under correct illuminating conditions and, apart from slight improvements which may be obtained by small adjustments of the substage iris, the instrument is giving the best image of which it is capable at the chosen magnification. Note particularly that the substage iris should never be used to control the light intensity; misuse of the iris in this manner degrades the quality of the image. The light intensity should be controlled either by neutral density filters or the lamp rheostat.

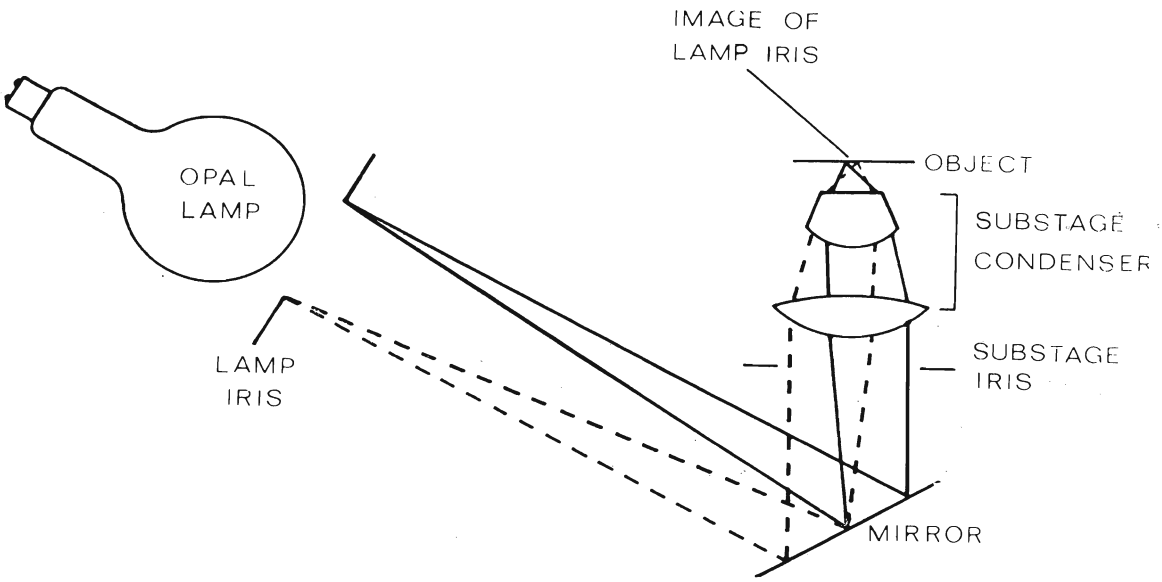

FIG. 3. Field-focused illumination, originated by Edward Milles Nelson (1851 - 1938).

To change to an 8 mm or 4 mm dry objective

(8) If the objectives are parfocal, rotate the nosepiece so that the 8 mm or 4 mm objective clicks into position, when the object will be approximately in focus; adjust the fine focus so as to obtain a sharp image. If the objectives are not parfocal, rack the body tube up before rotating the objective into position; then rack the 4 mm objective down carefully, watching from the side, until it nearly touches the slide and then rack upwards slowly until the object comes into focus. Remember that the working distance of a 4 mm objective is of the order of 0·5 mm.

(9) Close the lamp iris until the new smaller field of view is just filled with light.

(10) Remove the eyepiece, or insert the Bertrand lens, so as to inspect the back lens of the objective. Close and centre the substage iris and then open the iris to fill $\frac{2}{3}$ to $\frac{3}{4}$ of the objective aperture.

(11) Replace the eyepiece, or remove the Bertrand lens, and make any necessary small adjustments to the substage iris and fine focus. In making final adjustments to the fine focus, if the objective appears to be sensitive to cover slip thickness (8 mm to 4 mm dry objectives usually are), adjust the tubelength until the image of a point expands evenly on either side of focus.

2 mm oil immersion objectives

Usually, there is insufficient light from a diffuse lamp for proper operation with the oil immersion objective, and description of the procedure in this case is deferred until the consideration of Köhler illumination.

Difficulties with low-power objectives

If it is found impossible to fill the field of view of the 16 mm objective

with light when the lamp diaphragm is fully opened, adopt one of the following remedies—(a) move the lamp nearer to the mirror and re-focus the lamp diaphragm. This increases the size of source image in the field of view; (b) place a white diffuser over the mirror; (c) use a piece of ground glass or opal glass in the substage filter holder; (d) remove the top lens of the condenser; (e) use a negative (concave) lens in the substage filter holder to increase the effective focal length of the substage condenser; (f) remove the substage condenser and use the concave mirror as a condenser.

Setting up Aperture-focussed Illumination

In the method of illumination introduced by Köhler, a small-filament, high-luminance lamp with a clear bulb is used in conjunction with a lamp condenser. The lamp condenser appears uniformly illuminated with light, and together with the lamp iris is focused in the plane of the specimen by the substage condenser; the lamp filament is focused at the aperture stop (substage iris) by the lamp condenser, so that its structure is not seen in the field of view. This type of illumination is recommended for high magnification visual microscopy and for photomicrography.

Working instructions for setting-up with this type of lamp are given below, and the ray diagram is shown in Fig. 4.

(1) Centre the lamp filament on the axis of the lamp condenser. Do this by one of the following procedures—(a) If the lamp construction permits, rotate the bulb and inner housing to check centration, and make any necessary adjustments with the bulb-centring screws. (b) Project an image of the filament on to a screen from about 25 cm, and focus the lamp condenser from one extreme to the other. If the image moves laterally, adjust the bulb-centring screws. (c) Close the lamp iris until the diffuse area of light surrounding the filament image is not much larger than the image. Centre the image in this area by means of the bulb-centring screws.

(2) Arrange the lamp about 20 cm in front of the plane mirror and, if desired, tilt the microscope to a suitable working angle. Arrange the lamp so that the light beam strikes the centre of the plane mirror.

(3) Tilt the plane mirror until the light is travelling uniformly up the tube of the microscope.

(4) Place a specimen slide of reasonable contrast on the stage and, using a 16 mm objective, ×10 eyepiece and 160 mm tubelength, focus on the object.

(5) Close down the lamphouse iris, rack the substage condenser up almost to contact with the slide, and then rack down until the lamp iris comes into focus in the field of view. If necessary, centre the lamp iris in the field of view by very slight adjustment of the mirror, then open the lamp iris so that it just fills the field of view.

(6) Close the substage iris and observing its image in the microscope mirror, adjust the lamp and lamp condenser so as to obtain an image of the filament centrally on the substage iris. Alternatively, insert the Bertrand lens and adjust the lamp condenser so as to focus the lamp filament in the

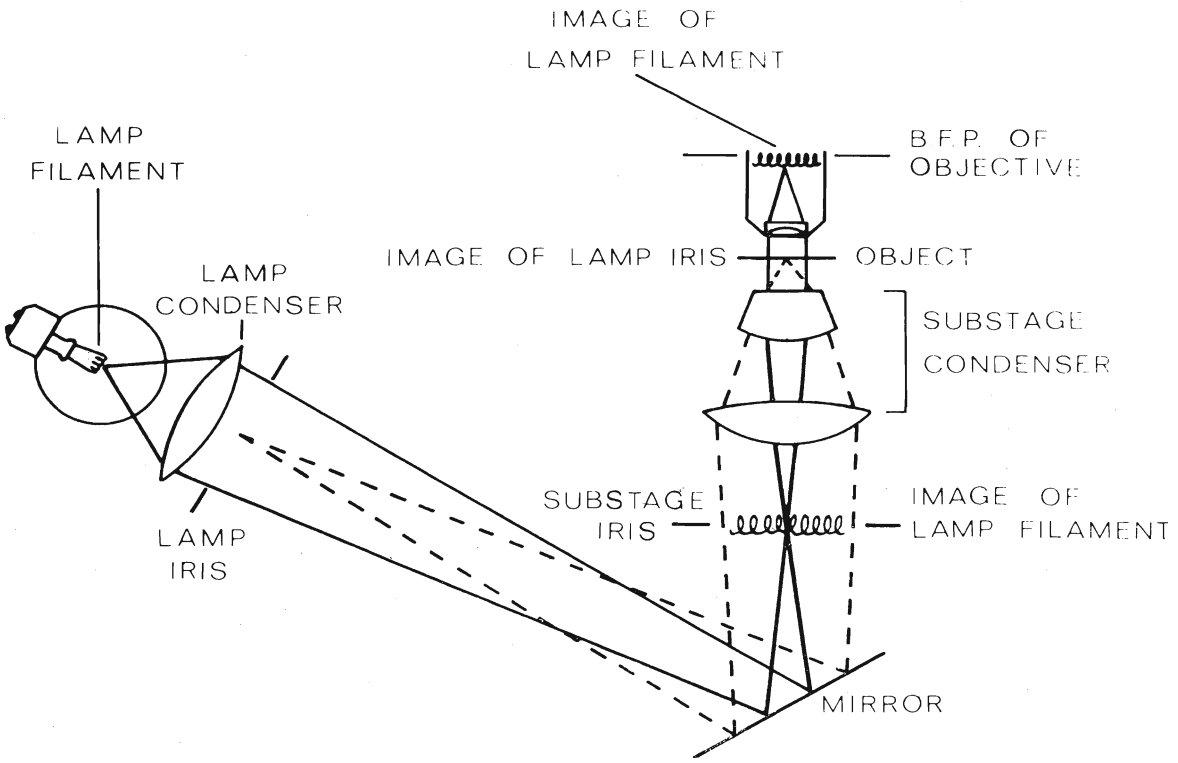

FIG. 4. Aperture-focused illumination, originated by August Köhler (1866 - 1948).

back focal plane of the objective. Centre the filament image by slightly tilting or swinging the lamp. Re-check centration of the lamp iris as in (5).

(7) Remove the eyepiece or use the Bertrand lens and centre the substage iris. Open the substage iris to fill $\frac{2}{3}$ to $\frac{3}{4}$ of the back lens of the objective.

(8) Replace the eyepiece. The remaining steps, if required, are as described in (6) to (11) in the instructions for setting-up with a diffuse light source.

To change to a 2 mm oil immersion objective

(12) Rack the bodytube up and remove the specimen.

(13) Place a large drop of immersion oil on the top lens of the condenser, being careful not to scratch the top surface of the lens. Replace the specimen and rack the condenser up to immersion contact.

(14) Focus on the specimen using a 16 mm objective and rack the condenser down until the almost closed lamp iris, or a needle point held in that plane, comes into focus. Open the lamp iris slightly.

(15) Rack the body tube up and rotate the oil immersion objective into position. Place a medium size drop of oil on the top of the coverslip and, watching from the side, lower the objective to immersion contact and a little below the expected focus position (working distance $\sim$ 0·15 mm). Rack slowly upward to the focus position with the fine adjustment. This initial focusing can be made easier by partially closing down the substage iris so as to increase specimen contrast.

(16) Close the lamp iris until the new smaller field of view is just filled with light.

(17) Remove the eyepiece, or insert the Bertrand lens, so as to inspect the back lens of the objective. Close and centre the substage iris to fill $\frac{2}{3}$ to $\frac{3}{4}$ of the objective aperture.

(18) Replace the eyepiece, or remove the Bertrand lens, and make any necessary small adjustments to the substage iris and fine focus.

(19) Remove the immersion oil immediately after use by two or three applications of xylol on a lens tissue.

Difficulties with low and high power objectives

If the field of view of low power objectives cannot be filled with light, adopt one of the remedies already given.

If the back lens of high power objectives cannot be filled by the image of the filament, move the lamp further away until the filament image spans the apertures both of the condenser and the objective.

Examination of interference figures (conoscopic observation)

When a Bertrand lens is inserted in the bodytube, to examine crystal interference figures in the back focal plane of the objective, it is obviously undesirable to see filament structure. In this case, insert a diffuser in the substage filter holder to give more uniform illumination at the aperture stops.

Alternatively, place the diffuser in the lamp filter holder or use a high-luminance diffuse lamp, and focus the illuminated diffuse surface in the substage aperture to give large-source Köhler illumination.

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THE QUEKETT MICROSCOPICAL CLUB

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THE QUEKETT MICROSCOPICAL CLUB (so called to emphasize the informal atmosphere of what is nevertheless a Learned Society and listed as such in a survey published by the Joint Committee of the Royal Society and the British Academy) was founded in 1865 to provide facilities for the friendly exchange of ideas between people interested in microscopy. Whilst the Club was originated and has always existed mainly for the benefit of those who take an interest in microscopical research as a means of intelligent recreation, all classes of workers with the microscope are most heartily welcomed. New members interested in particular branches of microscopy can usually be put in touch with other workers in the same subject.

The Club is fortunate to number among its members many professionals who have done much to further its interest and enhance its reputation, both at home and abroad.

It was initially a London Club, with the majority of its members living within easy reach of the City centre. With the disappearance of the smaller Microscopical Societies in the first half of the century, the Club has become a national one with a country-wide membership and an ever-increasing number of overseas members. The present total is over 500 members with about 90 overseas.

With its members spread out over such a wide area and only about 10% being able regularly to attend its meetings in London, the Club's publications provide a vital link to keep the majority informed of the Club's activities. The journal 'Microscopy' is published twice yearly and now spans up to eighty pages. In addition to distribution to members, it is acquired, either by purchase or journal exchange, by over 90 scientific societies and libraries. A News Bulletin is also distributed to members twice yearly.

There is a full programme of meetings throughout the year, some meetings being for lectures or the discussion of members' papers, and others being of an informal nature for the demonstration of objects and apparatus and for conversation. There are also occasional Saturday afternoon meetings for demonstration of slides and apparatus. An annual Exhibition of Microscopy is held every October. Details of the times and places of all meetings are published in the Club's programme, obtainable from the Secretary.

The Club has an extensive library bearing on microscopy and a large collection of slides, from both of which members may borrow. The Club also possesses a number of instruments for the use of members at the informal meetings. In the summer, excursions are arranged for Saturday afternoon visits to suitable collecting grounds. There is also a postal library service for out-of-town members.

The yearly subscription entitles members to all privileges of the Club, including free copies of the Journal and News Bulletin. There is no entrance fee.

Any person wishing to apply for election or who would like an invitation to a meeting of the Club should write to the Registrar at the above address.