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A symmetrical representation of the geometrical optics of the light microscope

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Summary

This paper provides an analysis of the functions of the optical components of the light microscope. Its novelty consists of a ray diagram in which the object is symmetrically related, either at a mirror plane or a centre of symmetry, to the optical components of the illuminating and imaging systems on either side of it.

Introduction

The object of this paper is to provide a simple explanation of the geometrical optics of the light microscope under the conditions of Köhler illumination by use of a ray diagram in which the illuminating and imaging optical components are arranged symmetrically on either side of the transmitting object such that they are mirror-images of each other. The ray diagram is equally applicable to reflected-light microscopy by folding it in the object-plane and in addition by the introduction of the requisite 45° beam-splitter normally situated in the optical space between the back focal plane of the objective lens and the primary image plane.

The illuminating and imaging optical components are represented as simple thin lenses; the focal length of the lamp-collector lens is made equal to that of the eyepiece and the focal length of the condenser lens is made equal to that of the objective. This situation is rarely, if ever, encountered in practice; the focal lengths of the imaging lenses, the objective in particular, are very much smaller than those of the illuminating lenses. However, the ray diagram serves a very important purpose: it places the object at the centre of the optical train and avoids the problem of 'cutting-off' portions of the ray-paths as they pass from the object and thence through the objective and eyepiece – a problem that can otherwise be avoided only by drawing the primary image and eyepiece on an inconveniently large scale. In my view it is this problem which detracts from, rather than facilitates, an understanding of the geometrical optics.

The symmetrical arrangement of lens components also emphasizes the symmetry of the 'aperture' or 'illuminating' and 'field' or 'imaging' series of conjugate planes and the respective diaphragms which lie in them. The functions of these diaphragms are explained below, but it is immediately seen from the ray diagram (Fig. 1) that the illuminated field diaphragm is conjugate with the eyepiece diaphragm, and the illuminating aperture diaphragm is conjugate with the objective diaphragm. The single ray diagram also avoids the artificial distinction between 'illuminating' beams or rays and 'image-forming' beams or rays which are frequently represented on separate diagrams (Delly, 1988; RMS, 1989; Bradbury, 1990). The artificial distinction is further compounded, particularly in books on polarized light microscopy, by the use of 'orthoscopic' and 'conoscopic' ray diagrams (Hallimond, 1970; Hartshorne & Stuart, 1970). It was always a source of confusion to me as to how the light paths in a polarized light microscope could suddenly change by simply removing the eyepiece or flipping in a Bertrand lens! The confusion is probably general. In most cases the authors draw attention to the artificiality of the distinction, but the problem of representation still remains.

Description of the ray diagram (1): the illuminating system

The filament is represented at the left-hand side of the diagram as a wavy line situated at a distance greater than the focal length of the lamp collector lens. Each point on the filament emits light in all directions, but of all this light we need consider only that portion that passes through the illuminated field diaphragm, situated in the back focal plane of the lamp collector lens. (The terms back and front focal planes are used in relation to the direction of light passing through the lens, back for the light passing out of the lens, front for the light passing into the lens.)

(i) Consider first the light radiating out from each point on the filament: three such points are indicated, a_o, b_o, c_o. The cone of light diverging from the axial point b_o is collected by the lamp collector lens and focused to b_1. Of all

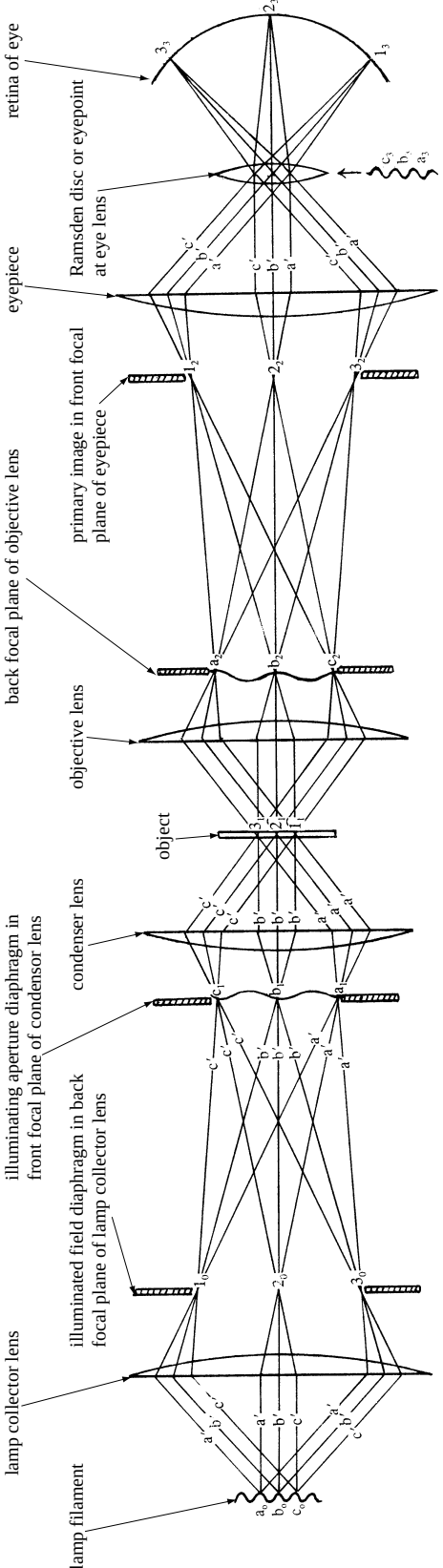

Fig. 1. Symmetrical ray diagram of the light microscope, showing the object (i) in a reflection plane of symmetry with respect to the optical components of the illuminating and imaging systems and the illuminated field series of conjugate planes and (ii) at a centre of symmetry with respect to the illuminating aperture series of conjugate planes. The rays $a'a'', b'b''$ and $c'c''$ arising from the separate points $a_0b_0c_0$ in the filament illuminate the whole area of the object as a series of parallel pencils and are focused in the illuminating aperture series of conjugate planes $a_1b_1c_1$, $a_2b_2c_2$, $a_3b_3c_3$. The parallel pencils of rays $a'b'c'$ arising from the whole area of the filament are focused in the illuminated field series of conjugate planes $1_02_03_0$, $1_12_13_1$, $1_22_23_2$ (primary image). The pupil of the eye or camera lens, focused at infinity, is situated at or near the Ramsden disc $a_3b_3c_3$ and produces the secondary image $1_32_33_3$ on the retina of the eye, film plane or CCD detector. In the case of reflected light the ray diagram is folded in the object-plane and a 45° semisilvered reflector is inserted in the optical space between the objective back focal plane and primary image plane. The asymmetrical element (the eye lens) in the ray diagram corresponds to the 'auxiliary' lens component of the reflected light illuminator.

this cone of light only the axial ray and two extreme rays are indicated by the letters b′. Similarly with point c_o just below b_o; the cone of rays, indicated by the letters c′, is focused to c_1 and so on. Points a_1, b_1, c_1 correspond to the image of points on the filament a_o, b_o, c_o which is represented by an (enlarged) wavy line and the magnification of which is given by the ratio of image-distance to object-distance.

The condition for Köhler illumination is such that the filament image is situated in the front focal plane of the condenser lens. Hence the rays diverging and 'continuing on their way' from points a_1, b_1, c_1 in the filament image are transmitted or projected as a series of beams or 'pencils' of parallel light that converge upon, and delineate, the whole illuminated field of the object. The important observation is that each point on the filament gives rise to a corresponding pencil of parallel rays that illuminate the whole field of the object; the axial point b_o gives rise to an axial pencil, and points a_o and c_o give rise to inclined pencils, all converging, as indicated, at the object. Clearly, the larger the area of the filament over which light is collected, the greater is the range of angles of incidence of all the pencils. Moreover, since each pencil originates from a point source, the light in each pencil is coherent. In short, Köhler illumination provides the conditions for the coherent illumination of the object. The coherence applies of course only to each separate pencil of rays, a′a′a′, b′b′b′, c′c′c′ and so on; there is no coherence between the pencils because they arise from different points on the filament.

(ii) Now consider pencils of parallel light emitted from the many point sources across the whole area of the filament. Three such pencils are indicated: the axial one (2) and the two highest angle ones (1) and (3) that pass inside the edge of the illuminated field diaphragm. These pencils of parallel light are focused in the back focal plane of the lamp collector lens; each point in the plane, e.g. 1_o, 2_o, 3_o, corresponds to the focus of a different pencil of rays emanating at different angles from the whole area of the filament. The rays diverge and 'continue on their way' to the condenser lens and then are focused to points 1_1, 2_1, 3_1 within the illuminated field of the object. Hence, each point on the object is illuminated by a parallel pencil of rays from the whole area of the filament; the centre of the object is illuminated by the axial pencil from the filament and points off-centre are illuminated by pencils from the filament at higher angles. This situation (ii) is simply the converse of that described under (i); the ray diagram could simply be reversed with the positions of the filament and object interchanged.

The functions of the illuminating aperture and illuminated field diaphragms are easy to see. The illuminating aperture diaphragm limits the area of the filament image and therefore limits the angular range of the parallel pencils of light incident upon the object. It therefore controls the numerical aperture of the condenser. The illuminated field diaphragm limits the angular range of the parallel pencils of light from the filament and therefore the area or illuminated field of the object. These diaphragms are adjusted in accordance with the requirements of the imaging system as described below.

The ray diagram for the illuminating system closely follows that drawn in August Köhler's paper (Köhler, 1893) except that, for reasons which are not clear, Köhler drew the pencils of rays from the filament, and those incident on the specimen, not precisely parallel but slightly convergent.

Description of the ray diagram (2): the imaging system

We adopt the same procedure as that above for the illuminating system.

(i) The light radiating out from each point in the object plane 1_1, 2_1, 3_1, etc., is focused to points 1_2, 2_2, 3_2, etc., in the primary image plane. Again, the magnification of the primary image is given by the ratio of the image distance to object distance. The primary image plane lies in the front focal plane of the eyepiece which is conjugate with the back focal plane of the lamp collector lens. The rays diverging from each point in the primary image plane are projected or transmitted by the eyepiece as a series of pencils of parallel light which all intersect at a (small) area called the eyepoint, exit-pupil or Ramsden disc. This is the point where the pupil of the eye should be placed, the eye relaxed and focused at infinity such that the parallel pencils are focused to form the secondary image, 1_3, 2_3, 3_3, etc., on the retina. The inclusion of the lens of the eye and retina appear to introduce an asymmetrical element to the ray diagram. However, they have their optical counterparts in the case of reflected light microscopy, as discussed below.

(ii) The pencils of parallel light passing through the whole field of the object a′a′a′, b′b′b′, c′c′c′, etc., are focused in the objective back focal plane to give a second filament image a_2, b_2, c_2, etc., conjugate with that formed in the condenser front focal plane. The light 'continues on its way' and is again focused by the eyepiece to give a third filament image at the eyepoint, exit-pupil or Ramsden disc which is conjugate with the filament. (A slip of tracing paper held at the Ramsden disc usually situated 10–20 mm above the eyepiece will reveal the Ramsden disc as a portion of the filament that contributes to it.)

The eyepiece diaphragm is clearly conjugate with the illuminated field diaphragm; the former is normally fixed and the latter is adjusted to match it, i.e. to appear at the edge of the field of view. Similarly the objective diaphragm is conjugate with the illuminating aperture diaphragm. Again, except for the case of special objectives with a variable numerical aperture, the former is fixed and the latter is adjusted to match it (in practice, and in order to

obtain conditions of optimum contrast, it is adjusted to be slightly smaller). These adjustments make heavy demands on the illuminating system, as discussed below.

The optical symmetry of the ray diagram

The ray diagram (Fig. 1) is symmetrical in two respects. First, the optical components in the illuminating and imaging systems may be regarded as mirror- or reflection-related across the object plane. However, if the successive inversions of the filament-image are taken into account (e.g. in the condenser front focal plane and objective back focal plane), the object can be regarded as situated at an optical centre of symmetry.

The symmetrical ray diagram shows the Ramsden disc as the same size as the filament and the primary image only slightly magnified. In practice the sizes of the primary image and Ramsden disc are (approximately) inversely related: the greater the magnification of the object the greater the demagnification of the filament. In this sense the microscope has two inversely related functions.

The diameter of the pupil of the eye should be equal to, or larger than, the diameter of the Ramsden disc, otherwise the field of view is reduced if the whole cone of rays is not intercepted. The latitude within which one can move one's eye (i.e. head) from side to side or to and fro from the microscope without impeding the full field of view clearly depends on how much larger the pupil is compared with the diameter, and the degree of convergence, of the rays at the Ramsden disc. The progressive improvements in eyepiece design are largely brought about by increasing the distance of the Ramsden disc from the eye-lens of the eyepiece (typically 20–25 mm, allowing the use of spectacles) and in decreasing the degree of convergence of the rays such that the location of the eye is not critical for the observation of a full field of view.

The symmetrical ray diagram shows the optical components in the illuminating and imaging systems as perfectly matched – which implies that for the ideal situation the condenser should be of the same design as the objective arranged in reverse and that each objective change should be matched by a corresponding condenser lens change. This situation obtains 'automatically' in reflected light microscopy because (as discussed below) the objective and condenser lenses are one and the same optical components. In transmitted light microscopy it was also formerly the practice to use, as a condenser, an identical objective 'in reverse' in order to exploit the limit of resolution of the objective – a practice now largely abandoned. In practice, the illuminating aperture and illuminated field diaphragms are adjusted to match the numerical aperture and object-field requirements of the objective lens. These can both vary by a factor of 10 or more – i.e. from the large-field (≈ 4 mm diameter) low-aperture ($\approx 0{\cdot}1$ NA) requirements of low-power objectives to the small-field (0·1 mm diameter) high-aperture ($\approx 1{\cdot}2$ NA) requirements of high-power oil immersion objectives. With such wide variations it is usually the case that the lamp collector lens is not large enough to fill the wide illuminated field diaphragm with light, nor, conversely, is the first lamp filament image large enough to fill the illuminating aperture diaphragm with light. One solution is to move the lamp assembly (filament, lamp collector, lens and illuminated field diaphragm) closer to, or further away from, the condenser for low and high power objectives, respectively – but this option is not available with 'modern' microscopes with built-in illumination systems. Alternatively, the effective focal lengths of the lenses, the condenser lens in particular, may be altered by the use of 'flip-in' lenses – but the use of these results in the diaphragms no longer being in their correct places. Hence, modern transmitted-light microscope design, with respect to the exact requirements for Köhler illumination, is something of a compromise.

Extension of the ray diagram for infinity-corrected objectives

Microscopes in which the objectives are computed for finite tube-lengths suffer the disadvantage that optical accessories – filters, beam-splitters and the like – introduced into the optical path between the back focal plane of the objective lens and eyepiece, affect the corrections of the objective lens. This arises because in this part of the optical path the rays from each point in the object plane 1_1, 2_1, 3_1 are convergent to each point 1_2, 2_2, 3_2 in the primary image plane. The aberrations that arise may be partially corrected by changing the optical tube-length (i.e. by adjusting the mechanical tube-length) but this facility is no longer generally available. The aberrations can be avoided (and the corrections of the objective maintained) only by inserting optical accessories into that part of the optical path in which the rays from each point in the object proceed as parallel pencils. The optical path between the eyepiece and the Ramsden disc fulfils this requirement – but only in the case, as shown in Fig. 1, where the eye is focused at infinity. In practice, the (virtual) image observed by the eye may be situated at any distance from infinity to the near point, the rays thus being divergent at the eye. However, a more practical consideration is that the optical path between the eyepiece and Ramsden disc is inconveniently short for the accommodation of optical accessories.

In the case of infinity-corrected objectives the object is situated in the front focal plane and the rays from each point in the object-plane 1_1, 2_1, 3_1 proceed as parallel pencils from the objective through the back focal plane to be focused by an additional lens – the tube-lens – at the primary image plane. Optical accessories may then be introduced into the optical path between the objective back

focal plane and the tube lens. The light in this region is sometimes loosely spoken of as 'parallel' as if the former could be extended indefinitely; properly speaking it is a region of parallel pencils (from each point in the object) diverging from the objective back focal plane. The position of the tube-lens must be such as to intercept these divergent pencils.

To maintain reflection-symmetry, the condenser lens should also be infinity corrected, with the object situated in its front focal plane and a tube-lens introduced between the illuminated field and illuminating aperture diaphragms. Again the introduction of optical components between the tube-lens and illuminating aperture diaphragm will not disturb the corrections of the condenser lens.

Extension of the ray diagram for reflected-light

Folding the ray diagram in the object-plane clearly shows that the condenser and objective comprise one optical component: the objective also acts as the condenser and their numerical apertures are automatically matched. Hence, an illuminating aperture diaphragm placed in the back focal plane of the objective lens (conjugate with the front focal plane of the condenser lens) would not allow an independent adjustment of the illuminating aperture of the condenser to a value slightly lower than that of the imaging aperture of the objective to obtain conditions of optimum contrast, as is the case for transmitted light. To provide for this situation the illuminating aperture diaphragm is normally placed, not in the objective back focal plane, but in a plane 'upstream' with respect to the direction of propagation of the light and conjugate with it. The filament $a_0b_0c_0$ is in such a conjugate plane. However, in order to avoid the practical problem of having a diaphragm situated in the plane of the filament itself, the latter is placed further 'upstream' and is focused, by means of what is sometimes called an auxiliary or relay lens (Galopin & Henry, 1972), into the plane of the illuminating aperture diaphragm. Hence in reflected light microscopy, the auxiliary or relay lens corresponds to the lens of the eye placed at or near the Ramsden disc. The important practical consequence is that the illuminated field and illuminating aperture diaphragms appear, in reflected light microscopy, to be in 'reverse order' to that in transmitted light microscopy.

In the case of infinity-corrected objectives the same criteria apply as for transmitted light. The 45° beam-splitter (or its equivalent) is situated in the 'parallel light' region between the objective back focal plane and tube-lens and a corresponding condenser tube-lens is placed in the illuminating system 'downstream' of the illuminated field diaphragm, i.e. closest to the microscope. In practice, reflected-light illuminators normally include such a lens, whether or not the objective is infinity-corrected (Galopin & Henry, 1972).

Further applications of the symmetrical ray diagram

The ray diagram may be used to demonstrate the principle of stereoscopic imaging (Hammond, 1996). A stop may be placed in the condenser front focal plane consisting of an opaque central bar and two plane (or circular) polarizing filters each side, their planes of polarization set at 90° or, in the case of circular polarizers, in opposite senses. The object is then illuminated only with inclined pencils of light, the pencils to right and left (i.e. $a'a'a'$ and $c'c'c'$) being plane polarized at 90° or circularly polarised in opposite senses. The monocular eyepiece is replaced by a binocular head that incorporates two polarizing filters in the eyepiece that are rotated such that they are crossed with the rays inclined to the left and right, respectively. Hence the right eye receives the inclined pencils $a'a'a'$ and the left eye receives the inclined pencils $c'c'c'$ which give rise to an orthoscopic stereo image. Rotation of the eyepiece filters by 90°, or inversion of their senses of polarization, gives rise to a pseudoscopic stereo image.

Finally, the ray diagram (Fig. 1) suffers from the defect common to all geometrical optics – namely that it does not provide an explanation of the physical basis of image formation; the all-important interactions of light with the object are wholly ignored. However, it can be reinterpreted, as it were, to illustrate the Abbe criterion for image formation, namely that at least two beams (normally the axial beam and two first-order diffracted beams each side) should pass the objective aperture. The light is then recombined in the primary image plane and it is this recombination of direct and diffracted light that is the physical basis of Abbe's theory of image formation.

Let the illuminating aperture diaphragm be closed down such that only the axial pencil of coherent light $b'b'b'$ passes to the object. The light is partially transmitted and partially diffracted by the object; the diffracted beams radiate out again as parallel pencils, the diffraction angles being determined by the spacings of the object-detail according to the conditions for constructive interference. Pencils $a'a'a'$ and $c'c'c'$ represent the largest angles, arising from the finest detail, which pass inside the objective aperture. All the pencils are focused in the objective back focal plane and the points of light a_2, b_2, c_2 constitute (part of) the diffraction pattern of the object. The light proceeding from these diffraction spots, which was separated by diffraction at the object, is recombined in the primary image plane. Hence the object-detail represented by 1_1, 2_1, 3_1 is reproduced in the primary image plane, 1_2, 2_2, 3_2.

Opening the illuminating aperture diaphragm admits coherent parallel pencils over a greater angular range, each pencil giving rise to its own set of diffraction spots (resulting in broadened 'spots' rather than points, in the objective back focal plane) and each set contributing to the intensity and resolution in the primary image. When a full cone of

light is admitted by opening the illuminating aperture diaphragm to equal that of the numerical aperture of the objective, the limit of resolution, as compared to that given by the narrow axial cone, is halved.

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