

Influence of light reflection at the objective in the quantitative measurement of reflectivity with the microscope

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Summary. When the microscope is used in the measurement of reflectivity there is a glare effect, due to internal reflections from optical parts, for which corrections have to be made. In the present paper these effects are measured in a new series of objectives for reflected-light microscopes, and it is shown that the factors involved are the number, curvature, separation, and blooming of the lenses, along with the conditions of adjustment of the microscope. For a given objective the conditions producing the smallest glare are: small area of specimen illuminated and measured, high numerical aperture of the illuminating system, and adjustment of the illuminator in such a way that the axis of the illuminating beams is slightly oblique. A theory of glare is outlined, and procedures for making the necessary corrections are described—by calculation and by graphical procedure. These corrections can be neglected when a sufficient number of graded standards is used.

THE quantitative measurement of reflectivity using the microscope can now be carried out with considerable accuracy. In order to make the best use of modern equipment and methods, systematic errors should be considered more than has been done previously. Effects due to light reflection in the microscope objective are of special importance in this respect. These effects were called 'éclairage parasite' by early French authors, such as Orcel (1936) and Capdecombe (1936). Stach (1955) calls it 'Eigenreflexion des Objektivs'. These authors also gave measured values for the effect. English authors have used the terms 'stray light' and 'flare', but it is pointed out by Bowie (*pers. comm.*) that 'glare' is preferable and that this usage is known among microscopists. Glare is light that reduces the image contrast and is, therefore, unwanted; hence we try to reduce it, as far as possible.

There are two effects to be considered here. The first effect is produced by the reflection of the incident light from the lenses of the objective towards the image plane; this happens before the light has impinged on the specimen. We shall express this effect quantitatively by the symbol ρ , which is the reflectivity of the whole objective. This effect has been referred to by Capdecombe (1936) as 'éclairage pro-

duit sans intervention de la surface étudiée', the latter being the surface of the specimen. The second effect is produced by infinite multiple reflections between the specimen and the objective, and Capdecombe (1936) describes it as 'éclairage dû aux réflexions multiples entre cette surface (specimen) et la face frontale de l'objectif'. That it is not quite correct to refer to the front surface only is proved by table II.

Measuring procedure and error correction. The simplest procedure is to use the quotient of the galvo-reading for the specimen G'_{sp} and for the standard G'_{st} multiplied by the true reflectivity R_{st} of the standard, thus obtaining a measured result $M'_{sp} = R_{st} \cdot G'_{sp}/G'_{st}$ for the reflectivity of the specimen. This result is more or less incorrect since we have not yet taken into account the effects of glare. The conventional procedure for eliminating the first effect is described, for example, by Hallimond (1953). This is easily done by measuring the glare while the front of the objective is in contact with a black cloth. The galvanometer reading C thus obtained is then subtracted from the readings for the specimen G'_{sp} and the standard G'_{st} , the adjustment of the whole optical assembly remaining unchanged; thus we have $M_{sp} = R_{st} \cdot G_{sp}/G_{st}$. But the error caused by the second effect remains. Bowie and Henry (1964) discussed the second effect, as well as the first, but not in any detail; they published an experimental curve of second-effect error obtained with bad optical conditions, simply in order to show the nature of the effect.

The amount of this error is $M_{sp} - R_{sp}$ which is the difference between the conventional measured result M_{sp} for the reflectivity of the specimen and its true reflectivity R_{sp} . It depends on three factors: the reflectivity of the objective ρ , as measured by the first effect of glare, the true reflectivity of the specimen R_{sp} , and the difference between the reflectivity of the specimen and that of the standard $R_{sp} - R_{st}$ (see p. 256 of the appendix). This has also been found experimentally by Leow (1966). The mathematical treatment (equations on p. 256 of the appendix) shows that the second-effect error relative to the true reflectivity of the specimen never exceeds $\approx 1\%$ when the reflectivity ρ of the objective is 1% . In many cases this small error can be neglected. But when the reflectivity ρ is larger, or when greater accuracy is required, both error corrections must be applied. In general this must also be done where the photometer is not very sensitive, with the consequence that the diaphragms have to be opened up. The correction for the second effect of glare can be made in three different ways, but first we must determine the value of the reflectivity ρ of the objective for the particular optical conditions.

First we can calculate R_{sp}^* using the measured result M_{sp} of the conventional procedure, the reflectivity R_{st} of the standard, and the value of ρ : $R_{sp} = M_{sp}/\{1 + \rho(M_{sp} - R_{st})\}$. We must distinguish carefully between specimen and standard; a standard is anything of which we know the reflectivity before starting the measurements being described.

Secondly we can use a graphical method. This procedure has been applied by Simpson (*pers. comm.*) and by Leow (1966). Before measuring the specimen the run of the second-effect-error curve is experimentally determined under the same conditions. For this purpose, the measured results M_{sp} of a series of known standards with reflectivities lying at suitable intervals are plotted against their true reflectivities. The run of measurements is made first with the lowest as *the* standard of reference for this experiment; measurements are then repeated using the highest as the reference. The measured results lie on a curve corresponding to the equations on pp. 256 and 257 of the appendix, which is shown in fig. 5. The difference between this curve and the (dashed) line of the diagonal gives the second-effect correction for any value of the difference between the measured result for the standard and that for the specimen being studied. The curve will vary with wavelength owing to the dispersion of the value ρ . Several known standards are required, but once the curve has been constructed it is quick and easy to use; exactly the same experimental conditions must, of course, be used for measuring the specimen as for constructing the curve. This method has the advantage that it includes all errors due to secondary effect of glare present in the measured result, whereas the theoretical approach has to neglect the minor factors.

Thirdly we can use a series of standards suitably graded in order that the second effect error may be negligible. For an accepted value of the tolerated second-effect error and the measured value of the reflectivity of the objective, we can calculate the reflectivities for the minimum number of standards that would be required to cope with a specimen of any reflectivity (fig. 3). At present we do not possess standards ranging throughout the scale of reflectivity, but fig. 3 can be used to assess the error for any given difference between the reflectivity of the specimen and that of the nearest available standard; if this exceeds the acceptable error then corrections must be applied, using either the first or the second method.

Symbols. The following symbols are used throughout this paper:

<i>OAS</i> , objective aperture stop	<i>Rec.</i> , receiver of photometer
<i>IAD</i> , illuminator aperture diaphragm	<i>St</i> , stage object
<i>IFS</i> , illuminator field stop	<i>sp</i> , specimen
<i>Oc.</i> , ocular	<i>st</i> , standard

PhS , photometer stop

R , reflectivity of stage object;

ρ , reflectivity of objective;

C , galvanometer reading correction for first effect of glare;

G' , galvanometer reading before any correction is applied;

G , galvanometer reading after the correction C has been made;

a , proportionality constant between galvanometer readings and reflectivities;

M'_{sp} , measured result for the reflectivity of the specimen (this contains the first-effect and second-effect error);

M_{sp} , measured result for the reflectivity of the specimen (this still contains the second effect error, which is given by $M_{sp} - R_{sp}$);

M'_ρ , measured result for the reflectivity of the objective;

σ_o , the half-angle of the aperture of the objective;

σ_i , the half-angle of the aperture of the illumination at the stage object.

Other conventions. The lamps, diaphragms, and stops all produce images, besides those formed of the stage object; images projected forward in the direction from lamp to ocular or photometer are distinguished by the symbol of the object imaged with one or more primes (') according to whether they are a primary, second, or third image; images projected backwards towards the lamp are shown with one or more bars over the symbol of the object imaged.

Ray paths in the instrument. Fig. 1 gives a schematic diagram of the ray paths; if we start at a point when a ray-bundle diverges, then an image of this point will be formed at the places where the same rays cross each other again, these places having diaphragms or stops situated at them. But we must also consider the angle of incidence of the light at any given place; this is shown in fig. 1 for the light impinging on the stage object, and it can be seen that this angle σ_o is limited by the size of the OAS , that is, by the distance separating the two limiting rays passing through this stop.

Following in turn each of the two ray-bundles shown, we note where they form images and where they limit an aperture. If we start with a point on the lamp filament L we follow what can be called the *illumination* path (dashed lines). This bundle forms an image L' in the IAD and then provides the limiting rays in the IFS ; it forms a second image L'' in the back focal plane of the objective OAS and then falls on the stage object. The other ray-bundle shown is called the (stage-) image-forming path, and we can best follow it (continuous lines), from the stage object St back towards the lamp. It forms an image \bar{St} in the IFS and then acts as limiting rays in the IAD before forming a second image $\bar{\bar{St}}$ in the lamp collector lens.

Now we can see that the image of the IFS lies in the stage, so that the size of the stage object illuminated is determined by the width of this stop. On the other hand, the maximum obliquity of the light

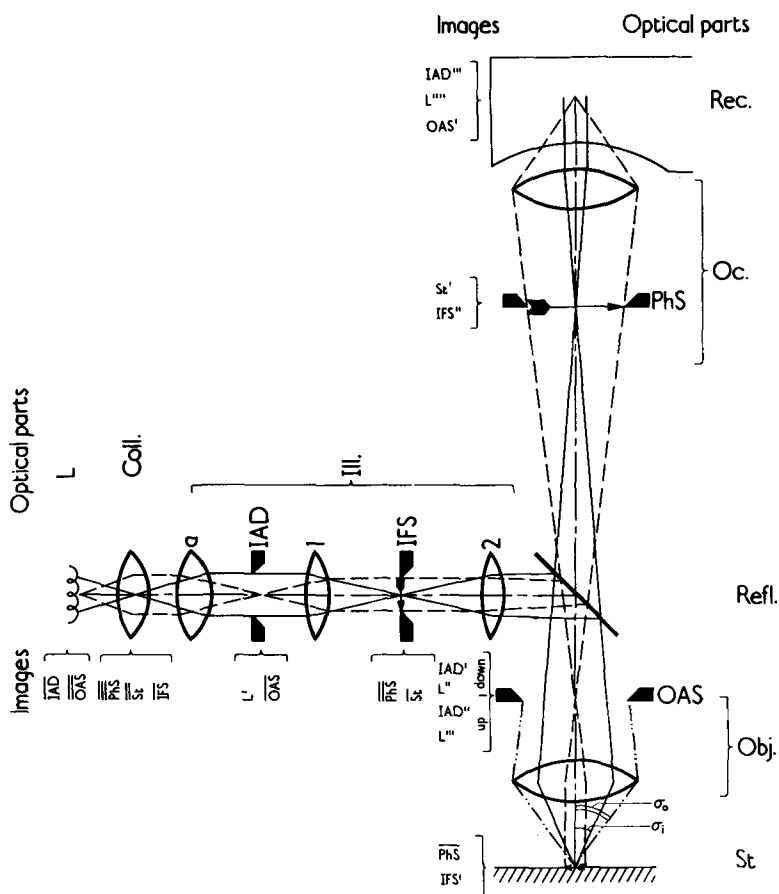


FIG. 1. Ray paths in the reflecting microscope, showing the paths to and from a point in the object as full lines, and the paths from a point on the lamp filament as dashed lines. L, light source; Coll., collecting lens; Ill., vertical illuminator; a, auxiliary lens; 1, 2, first and second lens or lens systems; Refl., reflector; Obj., objective; other symbols, see text.

impinging on the stage is determined by the other ray-bundle (continuous lines), which is limited by the *IAD*.

Let us now start again with the illuminated area of the stage object and follow the (dashed) illumination path upwards. We see that rays can be limited by the photometer (or ocular) stop *PhS*; thus we can receive all the light from the illuminated area of the stage object only when the *PhS* is sufficiently far open. If we insert the Bertrand lens, or

else remove the ocular, we observe the back focal plane of the objective; we see from fig. 1 that the image formed there is that of the lamp, and this is the correct arrangement of the illumination system. The dashed lines cross in the plane of the receiver, so that the image of the lamp, as reflected by the stage object, is formed there.

Starting now from a single point in the stage object, we follow the continuous lines and observe that they form an image of the stage object in the plane of the PhS ; a second image, not shown in the figure, would be formed in the retina of the eye, if the pupil of the eye were placed at the spot of the receiver.

The ray paths, from lamp to stage and then up to the receiver, are divided into sections, each of which is a portion of a right circular cone. The ends of the sections are formed by a stop (or diaphragm) or by its image; at one end of each section is an IAD stop or image, while at the other end is a PhS stop or image; the stop or image ending one section forms the beginning of the next section. The stops dividing the sections from each other are as follows:

First back image (\overline{IAD}) in the lamp.

Third back image ($\overline{\overline{PhS}}$) in the lamp-collector-lens.
Illuminator-aperture diaphragm (IAD).

Second back image (\overline{PhS}) in the illuminator-field stop.

First front image (IAD') in the back focal-plane of the objective, which is also the OAS .

First back image (\overline{PhS}) in the stage object St .

Second front image (IAD'') in the back focal plane of the objective after reflection by the specimen.

Photometer stop (PhS).

Third front image (IAD''') in the receiver.

With the glass-plate reflector the axis of the cone of light falling on the stage is usually perpendicular to the surface of the stage object, whereas with a prism reflector the axis of the cone is slightly oblique to this surface because the light comes from one half only of the objective aperture stop OAS . In order to have the complete image of the IAD in the back focal plane of the objective, it is necessary to displace slightly the IAD stop (not the reflector, which must always remain properly adjusted). This slight obliquity of the illuminating cone is an advantage in the measurement of reflectivity, as shown on pp. 253-4.

Note. The N.A. of the illumination and the obliquity of the illumination can be measured by means of the image of the illuminator-aperture diaphragm in the rear focal plane of the objective. This procedure is based on the fact that the distance between any point in the focal plane of the objective and its centre

corresponds to a certain direction of the ray coming from this point and impinging on the centre of the specimen. The quantitative interrelation between direction and distance from the centre is determined by focusing an image of the focal plane of the objective into the eyepiece by a Bertrand-lens. A micrometer scale is arranged in the eyepiece. Calibration is carried out by means of an apertometer or according to Mallard's method (with the aid of biaxial crystals having a known optic-axial angle). Details of these methods can be found in any suitable book on microscopy or polarizing microscopy (e.g. Rosenbusch and Wülfing, 1924, p. 351).

Reflectivity of the objective. The value obtained for the reflectivity ρ of the objective depends on the design of the objective, including the number of lenses, the curvature and separation of the lens surfaces, and the blooming of the lenses, and on the adjustment of the whole optical system between the lamp and the receiver.

The microscope manufacturer has to cope with the first of these two factors, whereas the user of the instrument has to pay special attention to the second. The manufacturer must make sure that the receiver does not obtain any stray light, and that no light is lost because of reflection or scattering at inner surfaces, rims of mounts, and contaminated glass surfaces. All sections in the microscope path of rays between the lamp and the receiver must, therefore, be limited by sharp-edged stops or by their sharp images, as it is by means of these that the measured area is delimited and the direction of the ray-bundles is determined. In order to obtain comparable measured results, values for the direction and the diameter of the bundles or rays impinging on the specimen should be known.

In determining the reflectivity ρ of the objective we first make a reading C with a black cloth held over the front of the objective. In order to scale this reading we then make a reading G'_{st} with a known standard on the stage under exactly the same conditions of adjustment $M'_\rho = C \cdot R_{st}/G'_{st}$. But this result is also influenced by an error due to the effects of glare. This error, $(M'_\rho - \rho)$, always has a negative sign. When the value of ρ is subsequently to be used in making the correction for the second effect by means of the appropriate equation, the accuracy of the measured value of ρ should at least be about $\pm 10\%$ of the true value.

This accuracy can most readily be achieved by using a medium to highly reflecting standard and taking the *uncorrected* galvanometer G'_{st} as denominator of the fraction. The relative error $(M'_\rho - \rho)/\rho$ has been calculated for different reflectivities ρ of the objective and taking as a reference standards with different reflectivities R_{st} . If a standard of reflectivity 100% could be used, the relative error in ρ would be zero, but even with $R_{st} = 50\%$, ρ must exceed 0.2 before its relative error exceeds 10%. In fig. 6 the minimum reflectivity of the standard necessary to ensure 10% accuracy in M'_ρ is plotted against ρ . A set of measurements was made on a new series of objectives for reflected-light microscopy specially designed to have minimum reflectivity ρ . In

order to approach the above-mentioned conditions an aluminium-coated surface was used as standard, the reflectivity of which had been increased to about 0.95 (i.e. 95 %) by means of a highly refracting layer of appropriate thickness. Examples of such surfaces, with reflectivity data, are given by Anders (1965).

Optical conditions affecting glare. In the previous section we have stated that the reflectivity of the objective depends not only on its manufacture but also on the whole adjustment of the microscope. The following tables illustrate the importance of the various factors involved, and reference may be made to fig. 2, in which the cone of light reflected from the objective is shown for various conditions of illumination. At the end of the section the optimum conditions for work are stated.

Table I shows the effect of leaving one lens unbloomed in different objectives, only one wavelength being used. It is seen that, in the same objective, a different value of reflectivity is obtained when a different lens is left unbloomed, this being especially notable in the $\times 80$ objective. The reflectivity is much reduced when all the surfaces are carefully bloomed. Table II shows the variation of the effect of blooming with wavelength.

Even larger differences of ρ for different wavelengths have already been demonstrated by Leow (1966). The variation is due to the particular process of blooming used by the manufacturer, and the reflectivity generally has its lowest value in green light. For making measurements in a single wavelength it is clearly advantageous to choose one in the green part of the spectrum, and $546\text{ m}\mu$ has been selected as standard by the Commission on Ore Microscopy of the International Mineralogical Association. The next three Tables give values for the reflectivity of the objective under different conditions of the optical adjustment of the microscope.

Table III shows the effect of different diameters of the illuminator-field stop (*IFS*). When the diameter of this stop is less than about 0.1 mm, diffraction effects arise round the edge of the image of this stop in the plane of the stage object and these produce uneven illumination of the measured area. In table III the illuminated area (column 4) is the image *IFS'*, while the measured area (column 2) is the back image (*PhS*) of the photometer stop. When we choose these images so that the ratio of the illuminated to the measured area is not more than about 2:1, we obtain the very small reflectivity shown in the lower line of column 5. On the other hand, where this ratio is larger, the reflectivity of the objective increases rapidly.

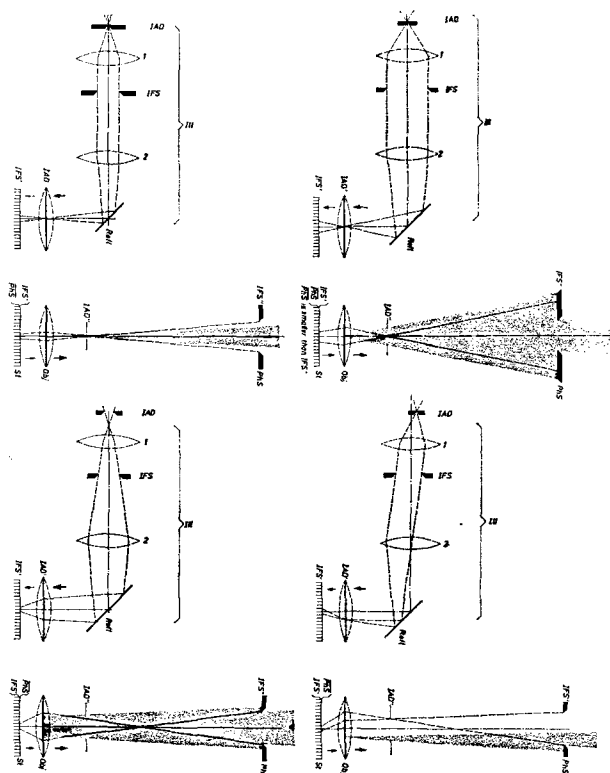


FIG. 2. Ray paths and light reflected from the objective under several conditions of illumination. Dashed rays as in fig. 1, but IAD' (OAS) in the lens; shaded cones, light reflected from the objective. *a* (top left), illuminator aperture diaphragm (IAD) closed down to a minimum, illuminator field stop (IFS) fairly small, photometer stop (PhS) just large enough to accept all light reflected by the (small) illuminated area of the stage object; the narrow cone of light reflected by the objective all reaches the photometer. *b* (top right), IAD and PhS as in *a*, but IFS opened out, illuminating a larger area of the object and leading to a larger cone of light reflected by the objective; all the latter light passes the PhS , but part of the light from the stage object is cut off. *c* (bottom left), IFS and PhS as in *a*, but IAD opened out, giving a cone of light reflected from the objective of the same angle as in *a*, but with its apex well below the stage; all light reflected by both objective and stage object passes the PhS . *d* (bottom right), IAD , IFS , and PhS as in *a*, but the IAD has been displaced sideways, giving oblique illumination; all light reflected from the stage object passes the PhS , but only about half of that reflected from the objective.

The results of table III are illustrated by figs. 2*a* and 2*b*. In fig. 2*a* the illuminator-field stop (IFS) is opened only to such an extent that the light bundle incident on the stage object illuminates no more than the measured area (PhS). The cone of light reflected from the objective

towards the receiver determines ρ . Its profile is smaller than the aperture of the photometer stop (*PhS*), so that all of this light reaches the receiver. In fig. 2*b* the illuminator-field stop is opened further so that the ray-bundle incident on the stage object illuminates *more* than the measured area. Thus more light is reflected by the objective, and by the stage object, but, since the photometer stop remains the same as in fig. 2*a*, the amount of light received from the *stage object* remains the

TABLE I. Reflectivity of completely and partly bloomed objectives, measured with plane-glass reflector in monochromatic light of $\lambda = 550 \text{ m}\mu$. (Lens surfaces are numbered from the front backwards in columns 7 and 9.)

Objective		Area of stage object measured (diam. in mm)	Area of stage object illuminated (diam. in mm)	N.A. of the illumination $n \cdot \sin \sigma_i$	Reflectivity of the objective $\rho\%$		
Mag.	N.A.				completely bloomed objective	objective not bloomed on the surface indicated	
$\times 16$	0.35	0.025	0.20	0.063	0.19	(2) 0.70	(7) 1.86
$\times 40$	0.85	0.01	0.08	0.17	0.65	(2) 2.08	(1) 2.02
$\times 80$	0.95	0.005	0.04	0.33	0.21	(2) 0.61	(8) 8.40
$\times 100$	1.25	0.004	0.032	0.40	0.025	(3) 0.04	
(oil)							

TABLE II. Reflectivity of completely bloomed objectives for different wavelengths using a plane-glass reflector

Objective		Area of stage object measured (diam. in mm)	Area of stage object illuminated (diam. in mm)	N.A. of the illumination $n \cdot \sin \sigma_i$	Wave-length in $\text{m}\mu$	Reflectivity of the objective $\rho\%$
Mag.	N.A.					
$\times 16$	0.35	0.025	0.20	0.063	500	0.35
					550	0.19
					600	0.21
$\times 40$	0.85	0.01	0.08	0.17	500	0.63
					550	0.62
					600	0.78
$\times 80$	0.95	0.005	0.04	0.33	500	0.49
					550	0.21
					600	0.37
$\times 100$	1.25	0.004	0.032	0.40	500	0.045
					550	0.025
	(oil)				600	0.029

same too. The portion of the light ρ reflected by the objective, however, is increased relative to that reflected by the stage object, because the profile of the corresponding cone penetrating through the *PhS* is larger, though a portion of this cone is cut off by the mount of the photometer

TABLE III. Reflectivity of the objective for varying areas of stage object illuminated, the measured area remaining constant. Experimental conditions: plane-glass reflector, bloomed objective, $\lambda = 550$ m μ .

Objective		Area of stage object measured	N.A. of the illumination	Area of stage object illuminated	Reflectivity of the objective
Mag.	N.A.	(diam. in mm)	$n \cdot \sin \sigma_i$	(diam. in mm)	$\rho \%$
$\times 4$	0.1	0.1	0.015	3.4	2.34
				0.2	0.0115
$\times 8$	0.2	0.05	0.03	1.7	0.58
				0.1	0.0028
$\times 16$	0.35	0.025	0.063	0.85	2.16
				0.20	0.19
$\times 40$	0.85	0.01	0.17	0.05	0.010
				0.34	8.1
				0.08	0.62
$\times 80$	0.95	0.005	0.33	0.02	0.028
				0.17	1.85
				0.04	0.21
$\times 100$	1.25 (oil)	0.004	0.40	0.01	0.018
				0.136	0.29
				0.032	0.025
				0.008	0.005

TABLE IV. Reflectivity of the objective for varying numerical apertures (N.A.) of the illumination (with plane-glass reflector)

Objective		Area of stage object measured	Area of stage object illuminated	N.A. of the illumination	Reflectivity of the objective
Mag.	N.A.	(diam. in mm)	(diam. in mm)	$n \cdot \sin \sigma_i$	$\rho \%$
$\times 4$	0.1	0.1	0.2	0.015	0.011
				0.09	0.0026
$\times 8$	0.2	0.05	0.1	0.03	0.0028
				0.20	0.00028
$\times 16$	0.35	0.025	0.05	0.063	0.010
				0.275	0.00036
$\times 40$	0.85	0.01	0.02	0.17	0.028
				0.75	0.0012
$\times 80$	0.95	0.005	0.01	0.33	0.02
				0.80	0.017
$\times 100$	1.25 (oil)	0.004	0.008	0.40	0.005
				0.96	0.0008

stop. It can also be shown that the absolute size of the photometer stop influences this relation, but the explanation is rather complicated and will not be given here.

Table IV shows that the reflectivity of the objective is also influenced by the aperture of the illuminating beams, i.e. by the diameter of the

illuminator-aperture diaphragm (*IAD*). The reason for this is demonstrated by fig. 2c. In this figure the illuminator aperture is seen to be larger than in figs. 2a, b, with the result that certain rays coming from the edges of the illuminator-aperture diaphragm (*IAD*) penetrate obliquely through the illuminator-field stop (*IFS*) and impinge obliquely on the stage object. The light-channel between the objective and the stage object is now a section of a cone having its larger base towards

TABLE V. Reflectivity of the objective for oblique and for vertical illumination

Objective		Area of stage object measured (diam. in mm)	Area of stage object illuminated (diam. in mm)	N.A. of the illumination	Obliquity of illumination at the stage object	Reflectivity of the objective ρ %
Mag.	N.A.			$n \cdot \sin \sigma_i$		
× 4	0.1	0.1	0.2	0.015	0°	0.011
				0.015	1.5°	0.00025
× 8	0.2	0.05	0.1	0.03	0°	0.0028
				0.03	3°	0.00025
× 16	0.35	0.025	0.5	0.063	0°	0.010
				0.063	5°	0.0004
× 40	0.85	0.01	0.02	0.17	0°	0.028
				0.175	14°	0.0004
× 80	0.95	0.005	0.01	0.33	0°	0.02
				0.36	20°	0.0004
× 100	1.25	0.004	0.008	0.40	0°	0.005
				0.42	15°	0.004
(oil)						

the objective. The quantity of light impinging upon the stage object is considerably increased because it is contained in a larger spatial angle.

The same is true with the light *reflected* by the stage object and travelling through the photometer stop. The diameter of the cone that contains the light being reflected by the objective (shaded area) is larger than the diameter of the photometer stop. Therefore the light-rays at the edges of this cone are absorbed by the mounting of the photometer stop. When the N.A. of the illumination is increased, the light reflected by the stage object is increased also. The light reflected upwards by the objective is increased in the same proportion as long as the cone all passes through the *PhS*. When this stop is closed so as just to exclude some of the cone reflected upwards by the objective, then a further increase of the N.A. of the illumination leads to a reduction of the light relative to that received from the stage object. Under such conditions a large value of the N.A. of the illumination results in a reduction of the value of ρ .

Table V compares the effects of vertical and oblique illumination. Oblique illumination can be achieved with the glass-plate reflector by

slight displacement of the *IAD*, which results in the images of this stop being formed off the axis of the illuminator. Use of a prism reflector always gives this effect, and in this case the *IAD* always must be displaced. With oblique illumination the axis of the cone reflected upwards from the objective lies off the central axis of the microscope. As can

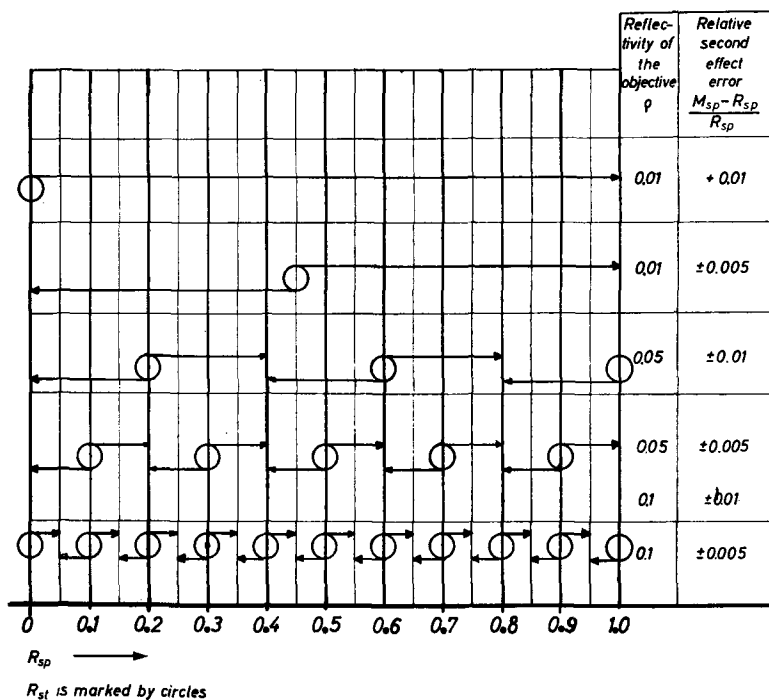


FIG. 3. Diagram showing, for a selection of objective reflectivities ρ and of maximum acceptable relative errors of a measurement, the range of specimen reflectivities for which standards of certain reflectivities (centres of the small circles) are satisfactory.

be seen from fig. 2*d*, this results in a large part of this cone being absorbed by the sides of the photometer stop; as a consequence the value of ρ is reduced.

It is to be noted that all previous considerations are based on the condition that the light impinges vertically or almost vertically on the stage object. If either the angle of the illuminating cone or its obliquity at the stage object becomes too great (greater than about 10°), the measurement will be incorrect. This danger arises especially when higher power objectives are used.

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Appendix

Mathematical analysis of the second effect of glare. In discussing the theory of the measurement of the reflectivity of a stage object we imagine a beam of unit intensity travelling downwards from the reflector of the microscope. The objective is considered to be represented by a single surface having a reflectivity ρ in both directions. Actually ρ consists of several components $\rho_1, \rho_2, \dots, \rho_m$, corresponding to the number m of the reflecting surfaces in the objective. These components are effective in the first order and in higher orders, and as multiple products. This fact will be neglected in this paper. Therefore the following analysis is to be considered as approximative only, but in spite of this it agrees well with experimental results.

A part (ρ) of the entering beam of unit intensity will be reflected upwards from the objective (fig. 4) and will enter the receiver of the photometer. The greater part ($1 - \rho$) will traverse the objective and fall upon the stage object. We can measure the reflectivity of the objective as described above by holding a piece of black cloth over the front of the objective and noting the galvanometer reading (C) obtained with the photometer. The light thus measured comprises the first effect of glare. This reading is, of course, a relative value and it depends on the sensitivity of the photometer; if we take a as being the proportionality constant, then $\rho = a \cdot C$. But C is the measure of the first effect of glare in the experiment, and this value should be subtracted from all other galvanometer readings G' , so that we have $G = (G' - C)$, and we always work with these corrected galvanometer readings G , except when determining the absolute value of ρ .

Let us next consider what happens to the beam incident on the stage object. Where R is the reflectivity of the stage object, the reflected intensity is given by $R(1 - \rho)$. On its way up this beam strikes the front surface of the objective whence a part $\rho R(1 - \rho)$ is reflected downwards again, while a part continues to travel upward to reach the receiver (fig. 4). The downward part forms a secondary incident beam and is again reflected by the stage object so that its intensity is now $\rho R^2(1 - \rho)$. It can be seen from fig. 4 that there is a series of 'reverberations' between the two surfaces stage object and objective, with part of the light escaping each time to reach the receiver. It is clear also that the magnitude of the effect will depend on the value of the reflectivity of the stage object since this absorbs some of the light at each moment of incidence. A stage object of zero reflectivity will absorb all the light incident upon it, and hence the second effect of glare will be zero. Likewise, a stage object of 100% reflectivity will reflect all the light in all the components of the incident beam, and so the second effect of glare will again be zero. For all stage objects with any other value of R , the second effect of glare will be non-zero, and its value will depend in some way upon R . The nature of this dependence is studied in the following pages, so that the method of making the necessary corrections can be properly understood.

First, however, let us consider the procedure of measurement already developed up to the point of obtaining galvanometer readings (p. 243). We know the value

of the reflectivity of the standard R_{st} and we have the two corrected galvanometer readings G_{sp} and G_{st} , so that we can write, $M_{sp} = R_{st} \cdot G_{sp}/G_{st}$, where M_{sp} is the measured result. Now this measured result contains within it the error due to the second effect of glare, and we must now examine this and see how to correct for it.

We have to sum over the various components reaching the receiver, and it is clear from fig. 4 that the reverberations give rise to a series,

$$(1-\rho)^2 \cdot R + (1-\rho)^2 \cdot R^2 \cdot \rho + (1-\rho)^2 \cdot R^3 \cdot \rho^2 + \dots = (1-\rho)^2 \cdot R / (1-\rho \cdot R).$$

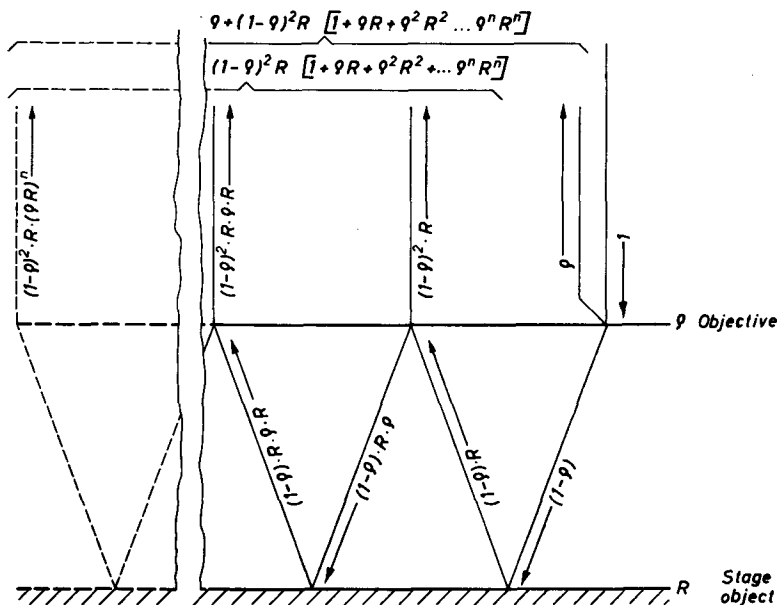


Fig. 4. Multiple reflections at objective and stage object.

This gives the total amount of light entering the receiver minus ρ , and substituting this value, both for the standard and for the specimen, into the equation for M_{sp} , we obtain for the error, after some rearrangement,

$$M_{sp} - R_{sp} = \rho \cdot R_{sp} (R_{sp} - R_{st}) / (1 - \rho \cdot R_{sp});$$

this may be divided by R_{sp} to give the relative error.

It will be obvious that when the reflectivities of the specimen and the standard are the same the error is zero, and that if we use a standard of very low reflectivity the error is proportional to $\rho \cdot R_{sp}^2$, and the relative error to $\rho \cdot R_{sp}$.

The equation may also be rearranged to give the true reflectivity of the specimen: $R_{sp} = M_{sp} / \{1 + \rho(M_{sp} - R_{st})\}$, which when R_{st} is very small compared with M_{sp} approximates to $R_{sp} = M_{sp} / (1 + \rho \cdot M_{sp})$. This is the equation for the family of curves above the dashed line in fig. 5. Leow (1966) has obtained experimental curves that agree fairly well with this theoretical curve; the dispersion of his

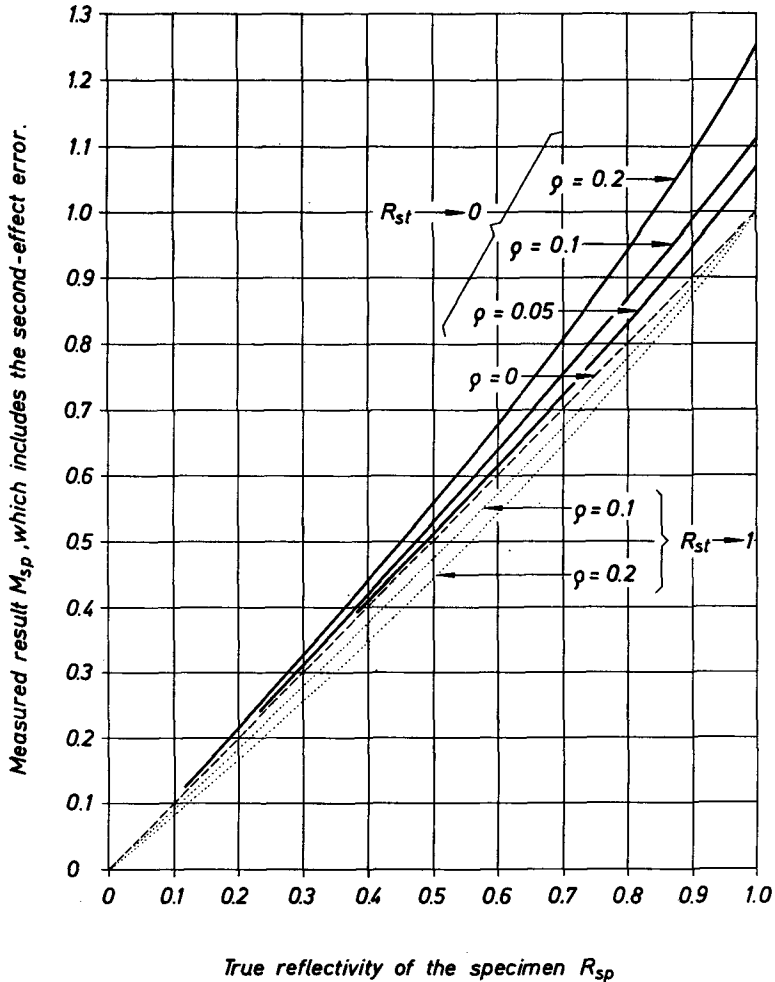


FIG. 5. Measured (M_{sp}) and true (R_{sp}) reflectivities of an object for several values of the reflectivity of the objective.

curves is due to the dispersion of ρ , the reflectivity of the objective, consequent on blooming.

Alternatively, if we use a standard of very high reflectivity, we obtain, in the limit when

$$R_{st} = 1, \quad M_{sp} - R_{sp} = -\rho \cdot R_{sp}(1 - R_{sp}) / (1 - \rho \cdot R_{sp}),$$

and

$$R_{sp} = M_{sp}(1 - \rho \cdot R_{sp}) / (1 - \rho);$$

the latter is the equation of the family of curves below the dashed line in fig. 5.

Measurement of the reflectivity of the objective. When measuring the reflectivity ρ of the objective according to the procedure described on p. 248, we get from the galvanometer readings the measured result $M'_\rho = C \cdot R_{st}/G'_{st} = C \cdot R_{st}/(G_{st} + C)$. Substituting $a \cdot C$ for ρ and $a \cdot R_{st}(1 - \rho)^2/(1 - \rho \cdot R_{st})$ for G_{st} , this leads to

$$M'_\rho = \rho \cdot R_{st}(1 - \rho \cdot R_{st})/\{\rho + R_{st}(1 - 2\rho)\}.$$

For a perfectly reflecting standard ($R_{st} = 1$) this reduces to $M'_\rho = \rho$, that is, there is no error; for a standard of poor reflectivity, the error becomes increasingly large.

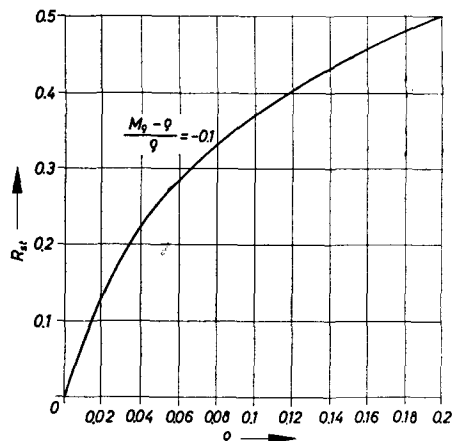


FIG. 6. Minimum reflectivity (R_{st}) of the standard to ensure a relative error not exceeding 10% in the measurement of the reflectivity ρ of an objective.

It is of interest to calculate how great R_{st} must be for a given relative error $E = (M'_\rho - \rho)/\rho$ and various values of ρ . The relation is readily obtained:

$$R_{st} = E + 1 - \frac{1}{2}E/\rho + \sqrt{\{(E + 1 - \frac{1}{2}E/\rho)^2 - (E + 1)\}};$$

in fig. 6, R_{st} is plotted against ρ for a relative error of 10% (i.e. $E = -0.1$, the error being always negative). This curve may be used to decide what reflectivity is necessary in the standard to ensure a relative error of less than 10% for any given reflectivity of the objective (a graph of R_{st} against M'_ρ would be strictly appropriate, but fig. 6 is accurate enough for its purpose); we see that for an objective with the very high reflectivity of 0.2, a standard with reflectivity greater than 0.5 will be adequate to ensure 10% accuracy in the determination of ρ .

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