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THE VISUAL DETERMINATION OF THE SIZES OF MICROSCOPIC OBJECTS BY DIRECT AND PROJECTION MICROSCOPY

by

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ABSTRACT

A general survey is given of the methods of image presentation and size determination by direct and projection microscopy. The relative merits of these techniques are discussed, in conjunction with such variables that must also be optimised for routine visual work.

The production and mounting of opaque disc test objects of known diameters in the range 0.2 to 1.2 microns is described and theoretical and experimental results available for the diffraction images of these test objects are discussed, including those of the author. Details are given of the construction of the direct and projection microscope systems, in particular the development and construction of a grainless screen for the latter.

Results are given relating the visual size to the true size of the objects over the whole size range under different operating conditions. In particular experimental work shows the dependance of the visual size upon the different viewing and sizing systems used, and conclusions are drawn regarding both the systematic and random errors produced by these systems.

PREFACE

As this thesis and its two subsidiary papers incorporate some work performed by others I should like to state the main items that were carried out by myself. They are :-

(1) (a) The production of the opaque disc test objects down to a diameter of 0.264 μ.

(b) The demonstration of the advantages of Aroclor, when used for the simultaneous mounting of transparent and opaque objects.

(2) The determination of the diffraction images of the opaque disc test objects down to a diameter of 0.264 μ.

(3) The development of the grainless screen for the projection microscope.

(4) The construction of the direct and projection microscope systems.

(5) The visual sizing of the opaque discs with:-

(a) The scanning block of the direct microscope.

(b) The filar micrometer eyepiece of the photoelectric micrometer microscope.

(6) The visual sizing of the opaque discs with :-

(a) The scanning block of the projection microscope.

(b) The calibrated wedge of the projection microscope.

(7) (a) The comparison of the sizing results obtained with the direct and projection viewing systems showing that they have identical systematic and random errors.

(b) The comparison of all the sizing systems considered in this thesis and an analysis of the factors which influence the systematic and random errors they produce.

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CHAPTER 1.

A SURVEY OF THE METHODS OF IMAGE PRESENTATION AND SIZE DETERMINATION BY DIRECT AND PROJECTION MICROSCOPY

1.1. Introduction

The magnifying action of a simple lens was first reported as early as the first century A.D. by Seneca, although the principles underlying refraction were not understood and applied until much later. The invention of the compound microscope is generally credited to Hans and Zacharias Janssen, at Middelburg, somewhere around 1590, but this is still a matter of dispute (see for example Disney et al (1928)).

Present-day optical microscopes, however sophisticated, still perform the same basic function, as the originals of the simple and compound types mentioned above, namely to produce a magnified image of an object. However for many years now it has been realised that resolution rather than magnification is the most important function of the microscope. This is especially so when a measurement of the visual size of the magnified image is required, the wave nature of light imposing a limit upon the smallest detail which may be observed.

In this chapter it is proposed to survey the various methods by which magnified images may be produced and then sized. The production of the magnified image or 'Image

Presentation' will be considered first in sections 1.2 to 1.10. to be followed by the visual sizing of the magnified image or 'Size Determination' in section 1.11 to 1.16.

1.2. Introduction to the Methods of Image Presentation by Direct and Projection Microscopy.

The magnified image of an object may be presented and viewed in either one of two general methods. It may either be viewed directly by placing the eye at the magnifier,- Direct Viewing, or be projected on to some form of screen and then viewed at a convenient distance - Projection Viewing. The merits of the two methods will not be discussed now, except to mention one important difference namely that an image viewed directly is virtual and that viewed by projection is real. The two methods will now be subdivided and considered individually as follows :-

Direct Viewing (Virtual images):

Monocular methods: section 1.3.

Binocular methods: section 1.4.

Stereoscopic methods : section 1.5.

Projection Viewing (Real images):

Front Projection methods: section 1.6.

Photomicrographic (projection) methods: section 1.7.

Back projection methods: section 1.8.

Grainless projection methods: section 1.9.

"Solid Image" projection methods: section 1.10.

1.3. Monocular Methods

In this the most simple form of magnified image presentation one eye is used to view the image. This naturally leaves the other eye unused. Barer (1953) has recommended that this eye should also be kept open, in order to reduce strain, but has suggested the use of a shade or a ground glass screen in front of it. Martin and Johnson (1958) have also recommended the occlusion of the unused eye.

1.4. Binocular Methods.

These, which include any method in which both eyes are used to view the same field, should permit a more natural use of the microscope. However opinions differ as regards their practical desirability. Payne (1954) states "The binocular microscope is more comfortable to work with and can be used for longer periods without fatigue or eye strain." While Chamot and Mason (1958a) state "The use of binocular microscopes requires that both eyes be maintained exactly centred with respect to the eyepoints. This necessitates holding the head and neck very rigid and causes greater muscular fatigue than does the use of a monocular instrument."

Preference therefore is not universal for either instrument, but varies according to the individual taste of each observer.

Binocular microscopes may be classified under two

headings namely 'Non-stereoscopic,' and 'Stereoscopic,' (which will be dealt with in the next section). Non-stereoscopic microscopes are mon-objective and present to the eyes two identical images. This is achieved by using a beam splitting device such as that shown in Fig.1.1., involving partial reflection and partial transmission at a semi-reflecting surface. However although there is no true stereoscopic effect the image appears more natural and a feeling of depth results.

1.5. Stereoscopic Methods

Stereoscopic vision in the binocular microscope is dependent on the formation of slightly different images in each eye. This may be accomplished in two ways:-

(a) by means of a separate compound microscope for each eye, directed at the same object from slightly different angles, as shown in Fig. 1.2. (Bi-objective stereoscopic microscope).

(b) By dividing and modifying the rays from a single objective so that different images are produced in each eye (Mon-objective stereoscopic microscope).

Whereas in (a) there are two separate objectives in (b) there is only one objective used in conjunction with an aperture splitting device, such as that shown in Fig.1.3.

1.6. Front Projection Methods

These include any method in which the image is projected on to an opaque screen.

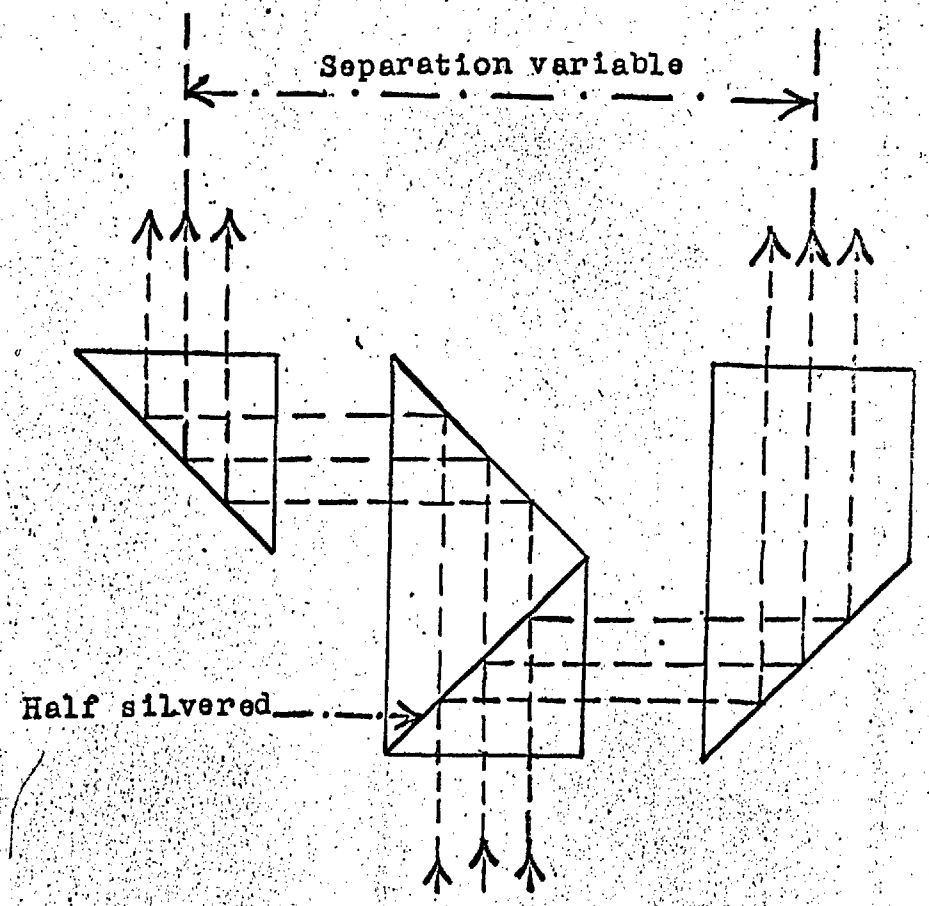

Fig. 1.1. Jentsch beam - splitting prism system.

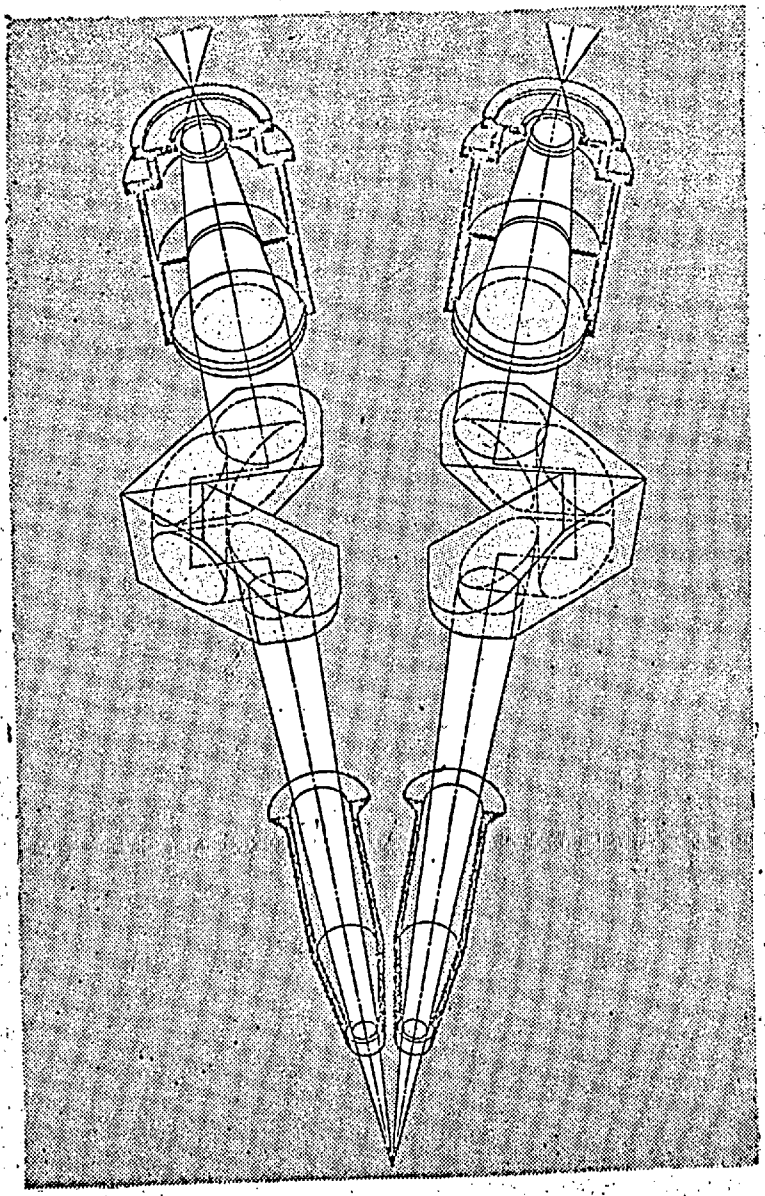

Fig. 1.2. Bi-objective stereoscopic microscope.
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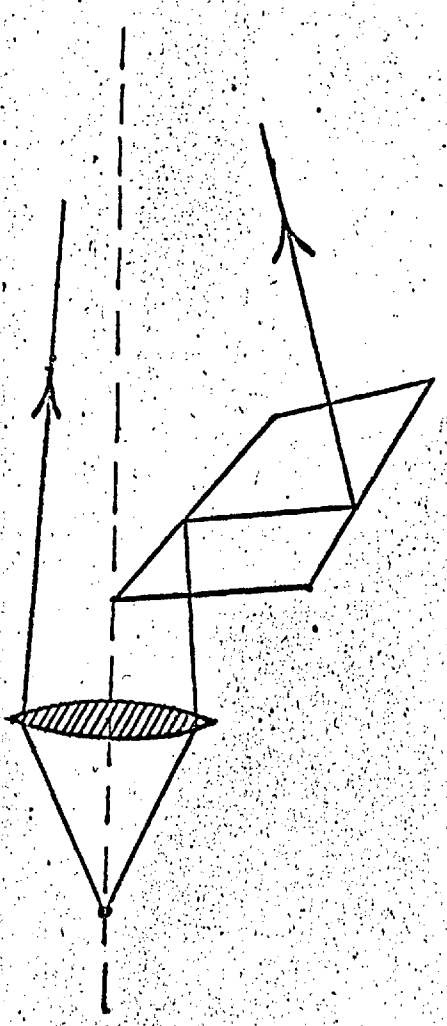


Fig.1.3. Wenham's aperture-splitting prism.

In straighforward front projection the screen is usually plain white with a smooth but non-glossy surface. For normal magnification this is placed about 25 cm from the microscope, under which conditions the microscope is between the screen and the observer.

1.7. Photomicrographic (Projection) Methods.

Photomicrography is a method of magnified image presentation that in fact incorporates front projection, the photographic film being the opaque screen. It has the great advantage of giving a permanent record of the object although it lacks the 'elasticity' of visual observation. One cannot, when looking at a photograph change the focus slightly to observe the effect unless a number of photographs at different foci are taken. On the other hand a photograph can be examined in detail at greater leisure than is possible with a changing object.

In photomicrography the image contrast in the photomicrograph can be improved during the development and printing processes. This means that that wavelength can be used which is best for resolution or for different absorption in the object.

Green (1921) and Loveland (1952) have both recommended the use of photomicrography as an intermediate stage in projection methods in general, the image first being photographed and then projected.

1.8. Back Projection Methods

In back projection the image is projected on to a translucent screen and then viewed from behind the screen. Under these conditions there is no physical obstruction of the view by the microscope as in the case of front projection.

Loveland (1952) recommends the use of a sloping desk-like back-projection screen, as the most convenient arrangement for protracted measurements. This arrangement is used in the Vickers Fifty-Five (Projection) Microscope, which advertises it as an "Inclined micro-crystalline wax projection screen for comfortable routine viewing."

1.9. Grainless Projection Methods

An important disadvantage of Projection Viewing is the phenomenon of 'graininess' in the projection screens. All projection screens have a more or less grainy sparkling appearance, which obscures the detail in the image, it is worse in back-projection than in opaque screens, but still present with both. The scale of the grainy appearance is considerably larger than that of the actual grain in the material, the magnitude of the effect increasing with decreasing numerical aperture of the illuminating beam. Even an ordinary ground-glass back-projection screen, no matter how finely ground has a very pronounced grain or sparkle.

Leifer, Spencer, Welford and Richmond (1961) have

suggested the use of two ground-glass screens placed with their ground surfaces almost in contact one being moved slowly in its own plane relative to the other. The sparkle and graininess are continually changing and are smoothed out by persistence of vision to give a perfectly grainless smooth screen. This arrangement is a development of an earlier suggestion by MacAdam and Taylor (1947).

1.10. 'Solid-Image' Projection Methods

A 'Solid-Image microscope has been designed by Gregory and Donaldson (1958) which presents the image as a solid in a luminous block. This is achieved by making the focal plane of the objective scan up and down through the depth of the object and then projecting the image on to a screen which is made to vibrate in phase with the focal plane of the objective.

The scanning serves to extract the information in depth from the object and the projection on to a vibrating screen of the image, reconstitutes it in dppth giving a 'Solid-Image' in space swept by the screen. The frequency of vibration greater than the fusion frequency for the eye, so that little or no flicker is observed. The further development of the 'Solid-Image' Microscope, has been described by Gregory (1960(a),1960(b) & 1961).

1.11. Introduction to the Methods of Size Determination by Direct and Projection Microscopy.

The determination of the sizes of microscopic objects

is extremely useful as an aid to identifications and also in the quantitative analysis of mixtures. The size of many natural objects is more or less characteristic and in the case of many substances such as textile and paper fibres may be very valuable as a means of identifying or of differentiating between materials of similar appearances but different magnitude. The size of artificial materials is often very important in governing their physical and chemical properties; size determination is commonly used in testing pigments, abrasive fibres, metals and alloys ~~sieves~~ and many other kinds of materials where fineness of structure is important.

However there is an inherent limit to the precision with which the size of microscopic objects may be determined. Apart from mechanical innacuracy in the apparatus used the limit is ultimately dependent upon the breadth of the diffraction image of the edge of microscopic structures, which is approximately equal to the resolution limit of the optical system. Hence the true position of any boundary which is to be measured could be uncertain to this extent. If a microscope objective of high resolving power is used the random error may be very small; for example ca. 0.2 μ for an objective of 1.40 N.A. This may be negligible in measuring the width of a textile fibre which is 25μ in diameter but it becomes much more important when pigment particles less than 1μ in diameter are measured. By

averaging repeated measurements the relative accuracy may be considerably increased; but the absolute dimension measured may nevertheless still be systematically displaced from the true value by a degree governed by the limitations of resolution.

The dimension of a microscopic object that is actually measured is usually its diameter. The term diameter may carry its ordinary meaning but since only in the case of an equidimensional particle (sphere or cube) does it have a single value, it almost always involves some attempt at averaging or combining the various different dimensions which characterize the shape of a non-equidimensional particle. Furthermore, the physical diameters of a particle are usually of indirect functional significance as compared with the surface or volume which may be computed from them, and any arbitrary system of expressing them by a single number should be such that representative values for surface or volume can be devised. The ideal diameter for particle size work might be defined as the diameter of a cube or sphere, with its surface (or its volume) equal to that of the original particle. Such a diameter may not be the same for surface as for volume, the discrepancy increases as the shape departs from an equidimensional one.

In practice the diameter that is normally taken is either Martin's or Feret's. The 'accidental' or 'horizontal' diameter suggested by Martin (1924) and illustrated in Fig.

1.4.(a) is the distance between opposite sides of the particle, measured on a line bisecting the projected area. Feret's (1931) diameter illustrated in Fig.1.4.(b) is the perpendicular distance between parallel tangents touching opposite sides of the particle. Other diameters include the mean projected diameter this being the diameter of a circle of equal projected area, and the diameter of a circle of equal perimeter. According to Chamot and Mason (1958(b), experimental comparisons of various proposed diameters have shown that the horizontal or Martin's diameter gives results in satisfactory agreement with those from the three actual dimensions of the individual particles, and the experimental simplicity of making a single measurement to be used without further computation has led to its being very widely employed in microscopical particle size work. Its validity decreases the less the particles approximate equidimensional shape, and corrections based on measurements of thickness may need to be worked out for dealing with needlelike particles. It should be emphasised that the horizontal diameter of a particle is useful only as a substitute for two or three measurements of the actual dimensions of that particle. It does not indicate how the particle will behave in a physical situation governed by true dimensions rather than artificially arranged ones.

Feret's diameter is not recommended according to

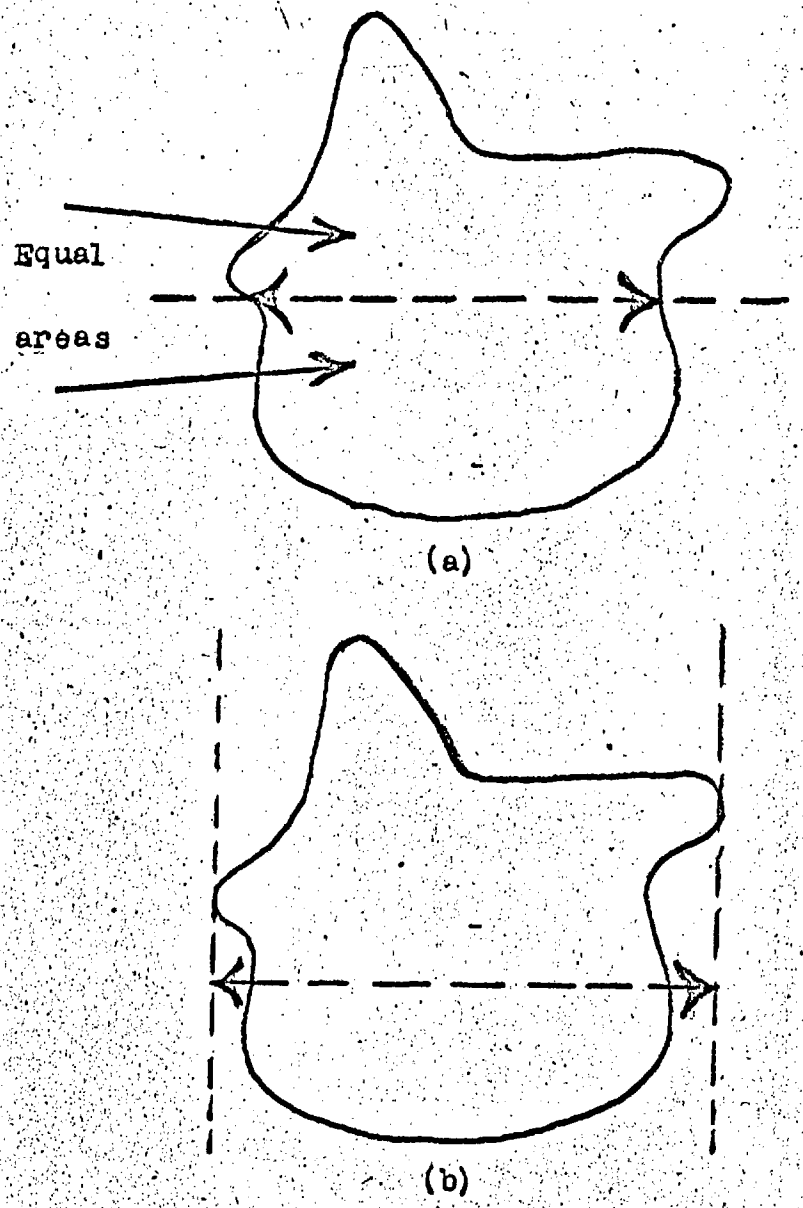


Fig. 1.4. - (a)Martin's diameter, (b) Feret's diameter.

Heywood(1946) because of the large errors introduced for elongated particles. However Walton (1948) has shown that Feret's diameter is equal to the diameter of a circle of equal perimeter provided that the particle profile is non-re-entrant, a condition which is usually satisfield. Other discussions of essentially the same aspects of particle sizing include those of Green (1927), Heywood (1937) and Kenrick (1940).

Most methods of visual sizing depend on the comparison of the object with a scale of known value. Therefore a microscopic standard of definite dimensions is required for the initial determination. For this purpose some form of stage micrometer is used, consisting of an accurately divided scale engraved on a suitable slide of glass or metal. High precision scales suitable for calibration have been produced by Brumley and Richardson (1950), using an Electro-optic crystal interferometer as a length measuring device.

The various visual methods for the sizing of microscopic objects by direct and projection microscopy may be sub-divided into the following groups:-

Graticule methods : section 1.12.

Sheared image methods: section 1.13.

Methods involving mechanical movement of the microscope or stage : section 1.14.

Depth measurement methods: section 1.15.

Counting methods: section 1.16.

1.12. Graticule Methods

One of the simplestways of measuring the size of an object, applicable at any magnification, is to view the object simultaneously with a high contrast linear scale, and make the necessary size comparison. The scale is calibrated using a stage micrometer. Simultaneous viewing of object and scale can be achieved by any one of the three following methods:

(a) By projecting the scale on to the object by means of the substage condenser.

(b) By mounting the scale at the focal plane of the eyepiece.

(c) By placing the scale on the projection screen.

The position of the graticules in all three methods is illustrated in Fig.1.5. Obviously methods (a) and (b) can be used for both direct and projection viewing whereas (c) can only be used for the latter. The three methods will now be considered in detail as follows:-

With method (a) by means of the mirror and the condenser, it is possible to project into the plane of the object lying upon the stage the image of a scale whose value has been ascertained. In this method described by Clendinnen (1910) both scale and object are magnified together and it therefore follows that no matter what may be the combination of objective, eyepiece and tube length empbyed, the value of the divisions of the scale image will remain unchanged, provided that the distance of thescale

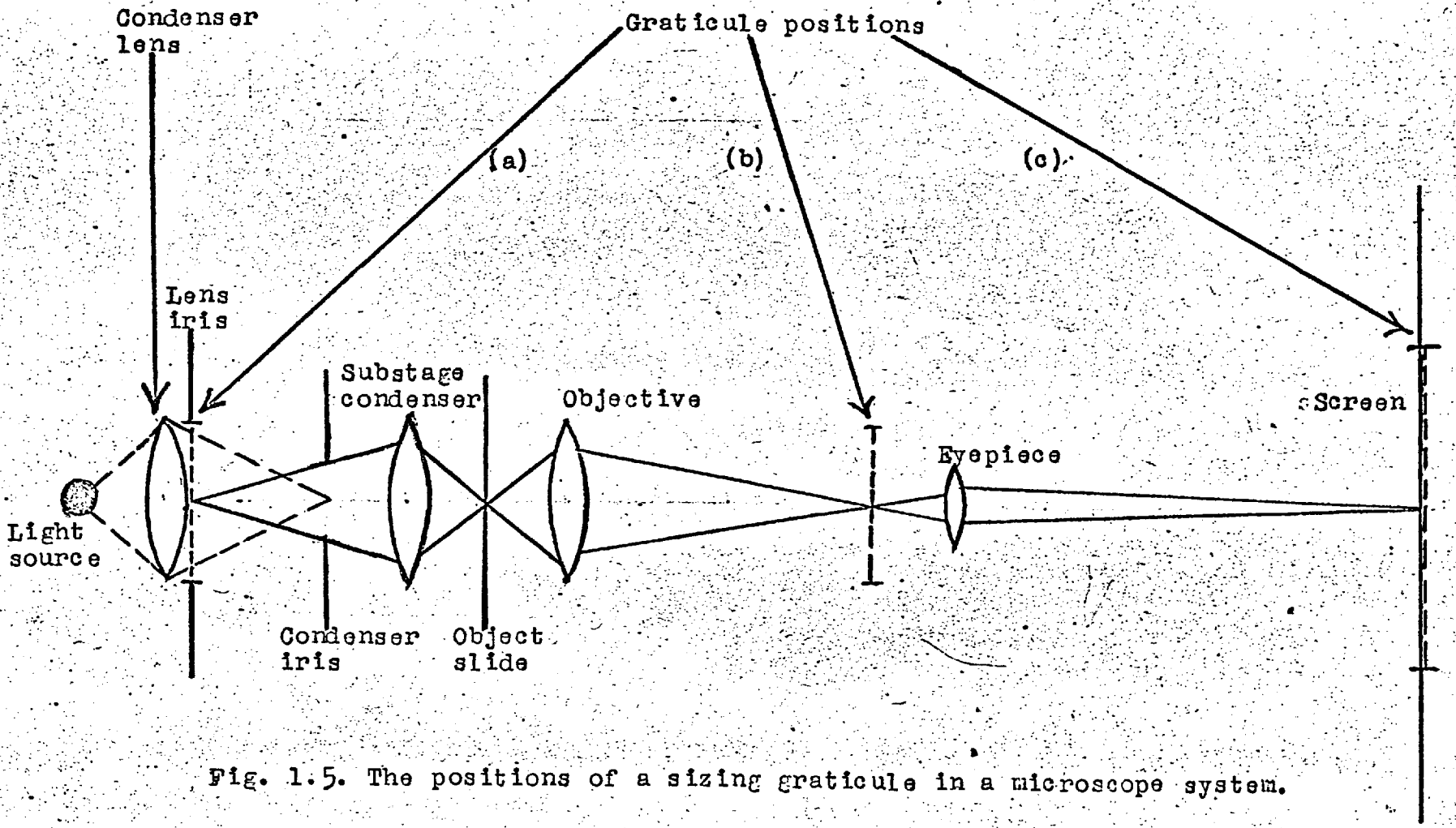
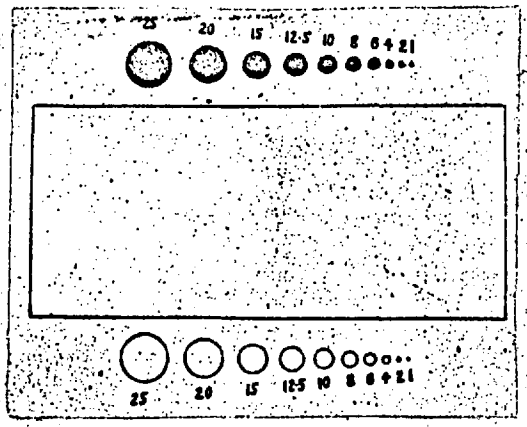


Fig. 1.5. The positions of a sizing graticule in a microscope system.

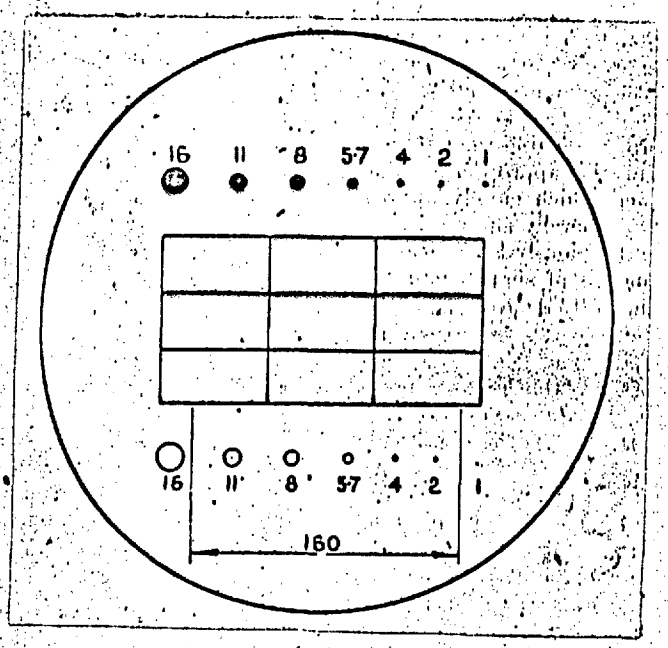
from the condenser is not altered. By varying the scale distance its value can be adjusted to convenient numbers of microns, with calibration against a stage micrometer. This method permits the use of a wide variety of scales or other external objects to be viewed simultaneously with the microscopic image. It is especially convenient when employing binocular microscopes, however it is necessary to ensure that the condenser is capable of giving a distortion free image of the scale used.

Method (b) renders object and scale simultaneously visible, by mounting the scale in the eyepiece on a reticle at its focal plane. By such an arrangement the real image formed by the objective is superposed on the scale and both are magnified by the eye lens of the eyepiece and seen together in the image. This is perhaps the most widely used method of microscopic measurement. The eyepiece scale is calibrated using a stage micrometer. As an alternative to a fixed scale a filar micrometer with a moving crosswire may be used, this giving measurements of greater precision at any point in the field of view. With individual objects the dimensions in any desired direction may be measured but with particulate specimens Martin's or Feret's diameters are normally taken, these being easily measured in the direction of crosswire movement. However direct linear measurements of this nature on a large number of individual particles are tiring and a particulate sample is generally classified

into a limited number of size groups. This was first done by Patterson and Cawood (1936) using a 'globe and circle' graticule shown in Fig.1.6.(a). This consists of a rectangle defining the counting field and ten opaque and transparent reference circles, which when used with a 2mm x 100 objective corresponded to diameters of 0.2 , 0.4,0.8, 1.2, 1.6, 2.0, 2.5, 3.0, 4.0, and 5.0 μ. With this graticule it was the mean projected diameters of the particles that were estimated and then grouped into the eleven size ranges. Fairs (1943) has described a graticule shown in Fig.1.6.(b) based on the Patterson-Cawood design using reference circles whose diameters increase in a $\sqrt{2}$ progression except at the smallest sizes and suggests that making the size interval any smaller, gives little improvement in sizing accuracy. May(1945) also describes a graticule with a $\sqrt{2}$ progression in circle diameter and having in addition a series of parallel lines whose separation from a given line increase in a $\sqrt{2}$ progression so that Martin's and Feret's diameters can also be measured. This is shown in Fig. 1.7. (a). Blacktin's(1946) graticule contained no reference circles and instead of the series of parallel lines consisted of a square counting field divided into a series of smaller squares, whose sides corresponded to 1,2,5, and 10 μ. Watson (1952) has used a much simplified version of the Patterson-Cawood graticule which contained only three reference circles, corresponding to diameters of 0.5,2 and 5μ

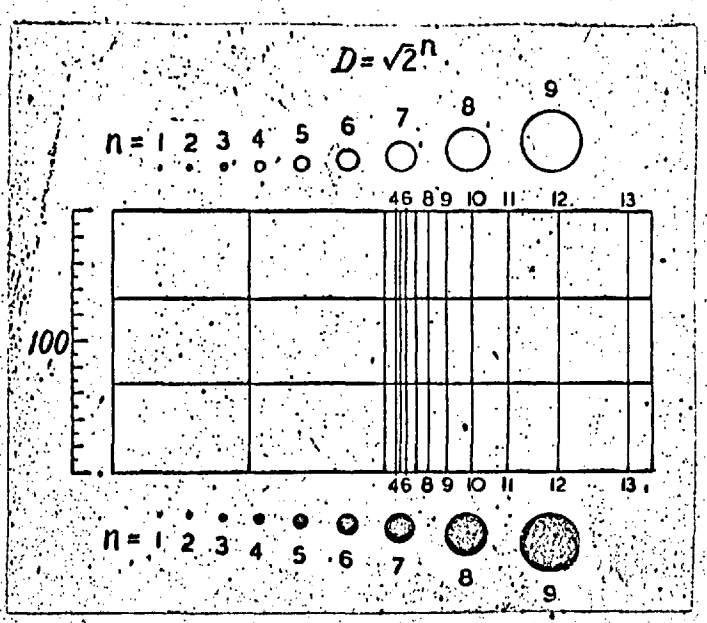


(a)

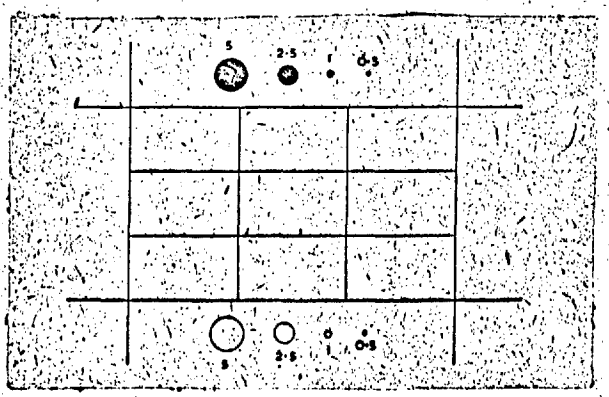


(b)

Fig. 1.6. - (a) Patterson and Cawood graticule,
(b) Fairs' graticule.



(a)



(b)

Fig. 1.7. - (a) May's graticule,
(b) Hamilton's graticule.

so that particles are classified into only four size groups. Hamilton et al (1954) modified Watson's graticule by changing the 2μ to a 2.5μ diameter circle and adding a 1μ diameter circle. In an investigation into the ease and accuracy of measurment with this graticule, shown in Fig. 1.7.(b), a Patterson-Cawood type and a line graticule similar to May's, they found that the observers expressed a definite preference for the simplified globe and circle type, on the grounds that it was easier to use, although the three types of graticule gave equal counting consistency. Holdsworth et al (1954) have showed that by standardizing techniques and comparing the results of regular cross-checks it is possible to maintain good agreement between well trained observers in different laboratories when using the globe and circle graticule. Watson and Mulford (1954) using a particle profile test strip were able to show that the sizing bias varied from individual to individual when using the globe and circle graticule and were able to give quantitative assessment of this bias.

In method (c) the object size is determined by direct measurement on the screen image. Fairs (1951) has described a method where a graticule is inscribed directly on to the screen and Loveland (1952) recommends that the graticule should be drawn on a transparent plate which can then be moved abouton the screen so that the outlines of the standard graticule areas can be superimposed directly on the image.

Circles, hexagons or other shapes appropriate to the objects being sized can be used as standard areas.

Where irregular areas, as are frequently found in biological or metallurgical studies are to be measured a series of graph-like cross hatchings may be superimposed upon the image, the square covered by the image of the object being counted to give its area. As an alternative to this rather tedious method a technique due originally to Delesse (1847) may be used. A sheet of uniform transparent material is placed over the screen image, and the outline of the structure whose area is required is drawn round and then cut out, the area of the structure being proportional to weight of the cut-out tracing. This method has been compared with the square counting technique by Mainland (1929) and with the use of a planimeter by Scammon and Scott (1927) all three investigators concluding that it was the easier and more rapid method. Mainland however, points out that for small areas of about 5 square mms. the cross-hatch method is both quicker and more accurate although for larger areas it rapdily loses efficiency. Loveland (1952) states that a planimeter, though slow may be used a a calibration check for other methods and advocates for the tracing technique the use of very thin Kodaloid as the transparent sheet material, the outlines being traced round with a hotpoint etching tool to eliminate the extra cutting-out process.

1.13. Sheared Image Methods.

The principle of these methods consists of producing two identical coplanar images of each particle, the centres of which are separated by a variable distance. The separation between the centres when the two images are arranged to touch is equal to the diameter of the images. The two images can be formed in various ways. Timbrell's (1952) arrangement consisted of a small mirror inclined at 45° to the optical axis of the microscope, which sent light into a horizontal eyepiece. This mirror was vibrated about a perpendicular to the optical axis at about 50 c/s, the light source flashing at the extremities of the vibration to give the two laterally sheared images. The magnitude of the shear could be calibrated in terms of the amplitude of the vibration. Timbrell suggested that repeated readings differ from their mean by less than 3% for 1μ particles and less than 1% for particles greater than 10μ. McGinn (1956) has used birefringent elements to give the two sheared images, although the magnitude of the shear is small. Baer (1960) uses the optical system shown in Fig.1.8. which is similar to the Mach-Zehnder interferometer, the mirror in one arm of the interferometer being rotatable. Two sheared images are thus produced, one by the fixed and one by the moving arm, the magnitude of the separation depending upon the tilt of the moving mirror.

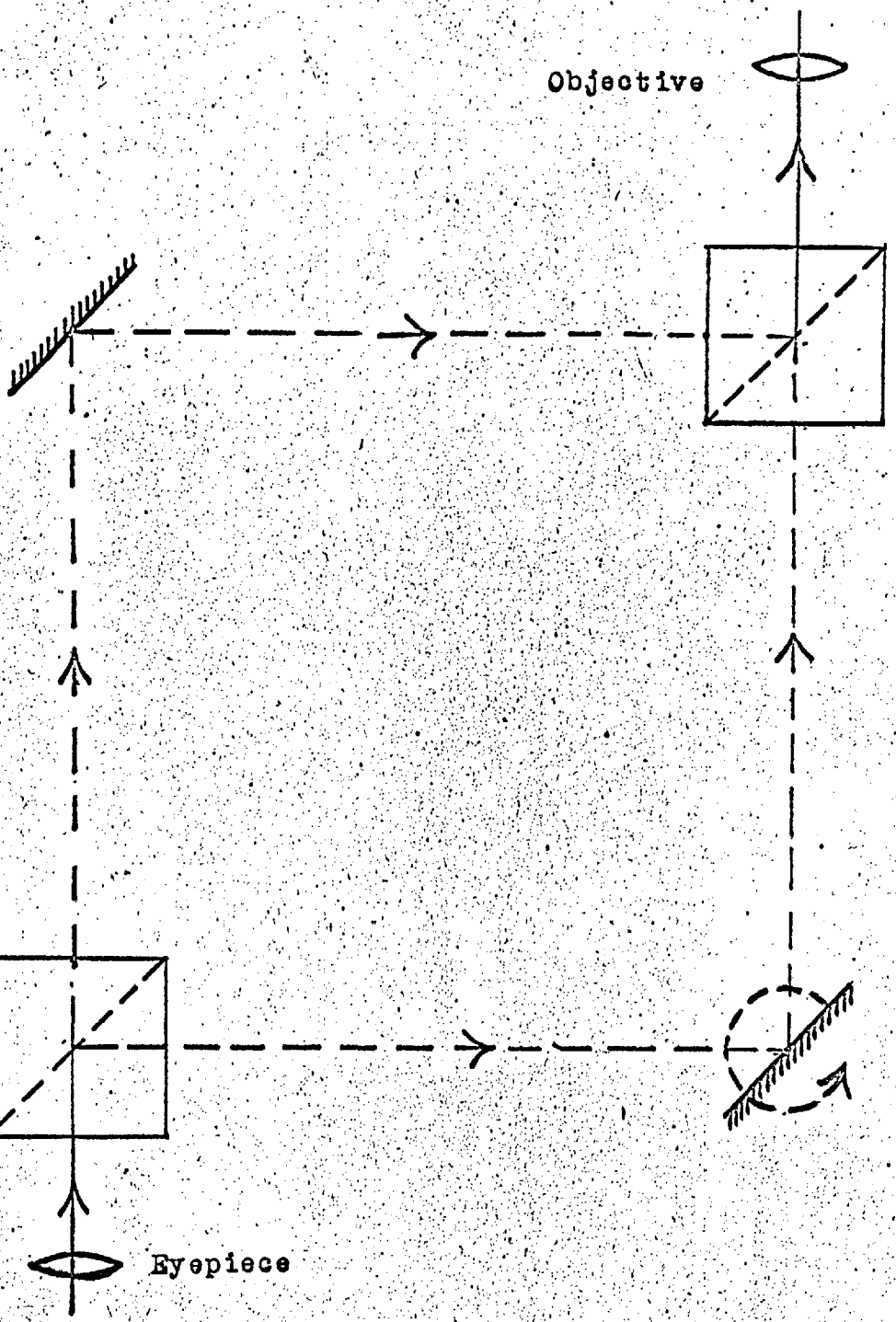


Fig. 1.8. Barer's sheared image method.

Dyson (1960(a),1961) has described a similar device based on the Mach-Zehnder interferometer shown in Fig.1.9. in which two identical prism blocks each consisting of one rhomboidal and one right angled prism cemented together with a half silvered interface, are used in each arm of the interferometer, this being inserted between the objective and eyepiece. Relative rotation of the two prism blocks gives a lateral shear between the two images from each arm of the system. This instrument is manufactured by Vickers-A.E.I. under British Patent No.901,319, and with it Dyson claims a setting error of about one tenth the size of the resolution limit of the microscope.

All the above instruments are best suited to measuring near spherical particles, it being possible to obtain integrated size distribution curves by setting the separation at a series of fixed values and counting the number of particles whose sizes lie above and below these values. A direct size distribution can be obtained from Timbrell's method by modulating the energising current which controls the amplitude of the mirror's vibration, with a low frequency square wave. It can also be obtained from Dyson's method as described by Dyson and Noble (1962) by mounting a thin glass plate on a pivot and placing it between the two prisms in such a way as to intercept only one of the two beams. If the glass plate is rocked to and fro through a small angle about the axis

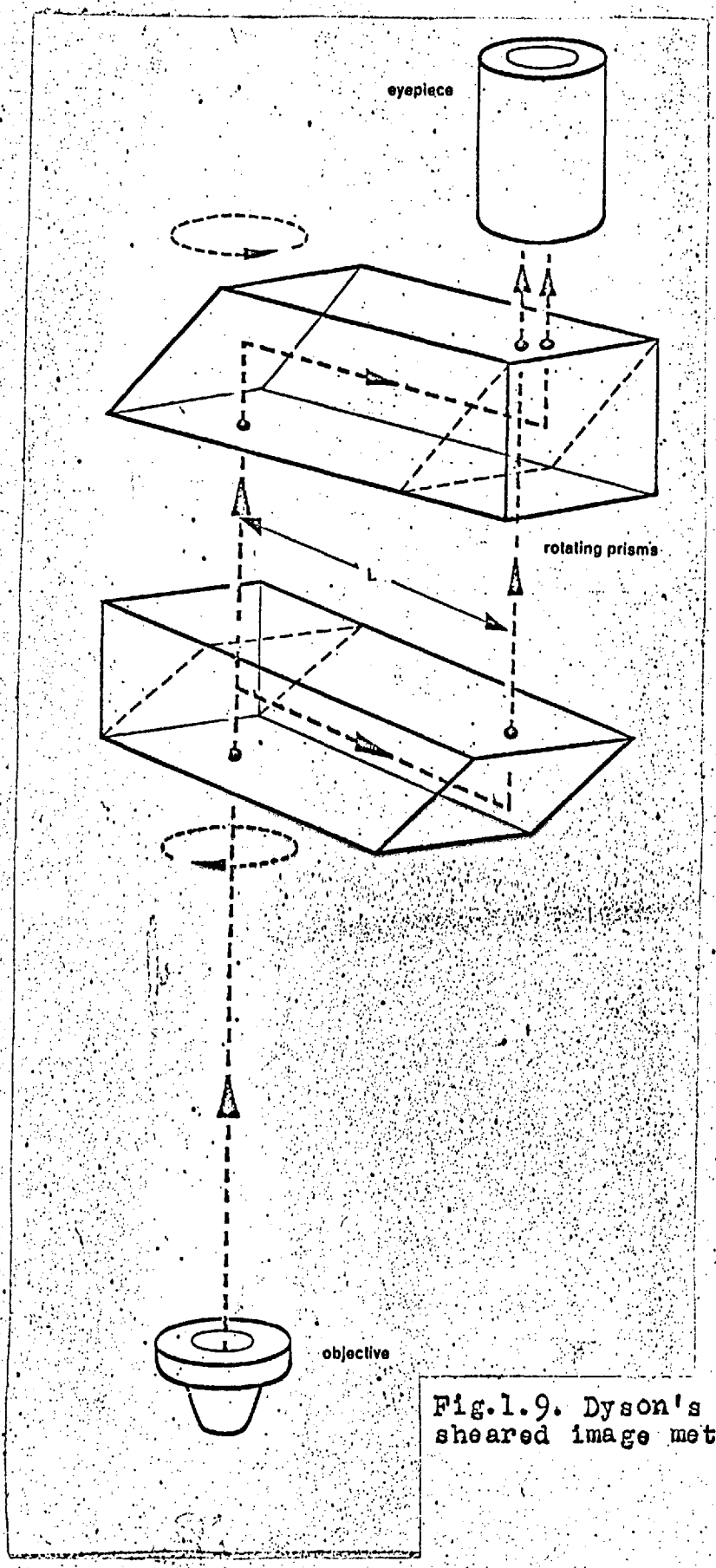


Fig.1.9. Dyson's sheared image method.

of the pivot, the beam passing through it is displaced. This desplacement adding to or subtracting from the shear given by the prisms themselves.

1.14. Methods Involving Mechanical Movement of the Microscope or Stage.

For objects which are larger than the field of view of the microscope, one of the simplest ways of measuring the object size is to use a travelling microscope, the microscope generally being traversed horizontally across the specimen. Crosswire settings are made on the edges of the object and the extent of the movement of the microscope and hence the size of the object is read off

on a micrometer drum. Alternatively a vernier mechanical stage can be used to move the object with respect to the fixed microscope.

If distances are to be measured cumulatively as in linear analysis a special recording micrometer is almost essential. Such instruments have a separate micrometer screw and scale for the measurement of each component distance. These recording micrometers, or integrating stages, are attachable to the top of the microscope stage. A type illustrated by Chamot and Mason (1958(c)) may be obtained with either four or six independent measuring spindles.

In these methods the accuracy is restricted by the

precision of the mechanical parts to about ten microns. Dunbar (1934) extended this technique to measure objects down to less than 1 μ in size by using a mechanical stage with a very fine movement, the motion being measured in terms of the movement of a system of interference fringes. He also adapted his microscope to measure depth to a similar degree of accuracy.

1.15. Depth Measurement Methods

If a microscope possesses a fine focusing adjustment which is graduated it may be used for measuring vertical distances. The distance between the top and bottom of an object (or between 'optical sections' at different levels) can be read on themicrometer screw. The value of the divisions on the fine adjustment is either marked on the instrument or may be calibrated by using for example a method suggested by Francon(1961(a)). This uses as a calibration reference an optically tested cover-glass of known thickness. Since for thismethod of measurment and calibration the refractive indices of the objects must be accurately known, it has been criticised by Galbraith (1955) who recommends the use of other methods wherever possible. Another criticism of this method is the lack of linearity of movement of most fine focus graduations. This can be corrected by calibrating the fine adjustment at the time of use using an interferometer as described by England (1957). This is done simultaneously in the

microscope attachment described by Dunbar (1934). Robins (1952,1954) has described a method of measuring the thicknesses of fine particles in the range 3 - 76μ in which a quartz lens is brought into contact with the object slide. By measuring the distance from the contact point of the lens and slide, the thickness of the particle can be calculated with about 5% error using the spherometer formula and knowing the radius of the lens.

Small differences in height of opaque specimens can be measured by using one of the many interference microscopes designed for the purpose, as reviewed for example by Hale (1958) or Krug et al (1964). These instruments are also applicable to thickness measurement on transparent speciments providing their optical path length compared with their surround does not exceed a few wavelengths, and that the various refractive indices are known. A very precise method of measuring small differences in height or thickness is the multiple beam technique due to Tolansky (1948 & 1960) where fringes are produced between the specimen and an optical flat. It is claimed that differences in surface height of as little as 5 A^{o} can be measured.

1.3.6. Counting Methods

These methods are applicable to particles near the limit of resolution, as well as to ultra-microscopic particles. The number of particles n in a known volume

V of a suspension of known concentration of particles C of known density ρ is counted, to give the "average particle diameter" $\bar{D}$, on the basis of either cubical or spherical particles. If the latter, $\bar{D} = \sqrt[3]{6CV/n\pi\rho}$

This method has been used mainly for particles near the limit of the optical microscope, which were assumed to be relatively uniform in size. Electron microscopy has shown that these submicroscopical materials are in fact often far from uniform, and not properly represented by an average diameter $\bar{D}$ which takes no account of this variation.

Dark-field illumination is commonly employed, because it gives better contrast, and because many more and much finer particles are revealed than by transmitted light. Drinker and Hatch (1936) give striking illustrations of the increased countsof dust samples with dark-field illumination. The dark field appears like the night sky, and may be produced by using either a dark-field condenser generally of the cardioid or Cassegrain type or the ultramicroscope described by Seidentopf and Zsigmondy (1903). In order to eliminate the effect of Brownian movement, either spray counting or photographic exposure using a xenon flash lamp, as described by Loveland (1952) must be used to 'stop' the motion. These dark field counting techniques can be used to size particles down to 5 mμ but suffer from the disadvantage that extraneous dirt

particles cannot be distinguished from the system under observation and that only under exceptional conditions is any shape information given. Their efficiency falls off if any coarse particles as well as the fine ones are present since those scatter large amounts of light, thus lowering the contrast and making the finer particles difficult to detect.

CHAPTER 2.

THE OPTIMISING OF WORKING CONDITIONS IN ROUTINE VISUAL MICROSCOPY

2.1. Introduction

Of all the methods of size determination described in Chapter 1 those in which measurements are made on the plane of the object lying perpendicular to the optical axis of the microscope, namely graticule methods (section 1.12.), sheared image methods (section 1.13.) and methods involving mechanical movement of the microscope or stage (section 1.14.), are finally limited in their accuracy (systematic error) by the way in which the eye assesses the diffraction image of the edge of the object whose size is required. Of the other methods, the depth measuring techniques described in section 1.15. are limited to sizing particulate specimens above 3 μ in size, with the exception of the multiple beam technique which is best adapted to the measurement of surface irregularities on an extended object, and the count technique described in section 1.16. is limited to determining the average size of a large number of similar objects. Thus for general visual work with the microscope it is fair to say that the limiting accuracy of the sizing process is set by the correct assessment by the eye of the geometrical position of the edge of the object whose size is required with respect to the diffraction image of the edge given

by the microscope. This applies both in the case of area or length determinations, an error in linear dimensions giving an error in area dimensions. Clearly information on the accuracy of the above-mentioned assessment is desirable. In particular the practical need is for the systematic errors for each sizing technique to be determined as a function of object diameter and form using known objects and well defined optical conditions. The relationships found could then be used to calibrate future measurements under the same conditions of similar objects of unknown diameter. Such an experimental programme would need to be enormous to cover all the variations in object form, scale, refractive index and transmittance, together with variations in imaging, viewing and sizing techniques, and optical and visual conditions, i.e. all the possible variations in working conditions in routine visual microscopy. Much can be gained however by using objects of simple form such as spheres, cylinders, strips and discs, the properties of such forms often approximating to those of specimens which need to be sized in practice. Further discussion on the choice and preparation of suitable test objects will be left until Chapter 3, while the rest of the present chapter will be devoted to the discussion of the other variables involved.

2.2. Image Presentation and Viewing Conditions

The main purpose of this section will be to discuss

the relative merits of direct and projection microscopy.

Many workers prefer projection methods although the American Society for Testing Materials (1951) only recommend these for particles above 2 μ in size. Dunn (1930) and Brown and Yant (1935) are unanimous in suggesting that projection methods offer significant improvements in sizing accuracy, the latter expressing their views as follows:-

"Direct visual microscopic determination of particle size distribution and number concentration of atmospheric dust is time consuming and trying to the eyes. By using the microscope to project images of the particle at high magnification on to a screen much of the eyestrain can be eliminated and particle size distribution results obtained with less effort and at a considerable saving in time. It also permits simultaneous observation of theimages by more than one person."

From the above it can be seen that preference for projection viewing is obviously due to improved comfort and convenience Loveland (1952) has emphasised the importance of these factors in protracted measurments, stressing their effect on both the observer and the results.

Many microscopists however feel that in projection microscopy much of the image detail is lost as compared to direct microscopy. Two reasons are suggested for this relatively poor performance of projection microscopes.

The first is the flatness of the image, there is an impression of solidity and reality to be obtained from the virtual image seen directly, even with a monocular instrument, which is lacking from a projected image; this is partly due to accomodation of the eye, projected back through the optical system of the microscope to view the object in different planes, and partly due to the continued use of the fine focus control. With a projection screen on the other hand, the eye cannot accomodate back through the system and although again the fine focus would be in use, it no longer gives the impression of moving back and forth in depth, it presents simply a changing picture; the surface of the screen is always present as an object in the visual field and provides a flat reference plane from which visual impressions cannot be dissociated, whereas the virtual image seen by direct viewing appears suspended in space with no reference surface other than the dark rim of the field stop.

The problem of flatness in projection viewing can be overcome by using the 'Solid-Image' microscope of Gregory and Donaldson (1958) described in Chapter 1, section 1.10. However the contrast of the 'Solid -Image' is relatively poor due to the presence of background illumination from the other planes in the luminous block.

The second reason suggested for poor performance of projection systems is the grain of the projection screen.

This problem has already been mentioned in Chapter 1, section 1.9., but will now be considered in full detail.

All scattering screens must have some graininess which has a structure at least an order of magnitude greater in scale than the wavelength of light, or they would not scatter at all, but it might be supposed that it still could be small enough to be un-noticeable under normal working conditions. In fact all screens have a very clearly visible graininess showing up as a kind of sparkle under some conditions, which obscures the finer structure of the image; it is worse in back-projection than in opaque screens but still present with both. The obvious answer is to magnify the image until the detail is much larger than the grain, but this cannot be done because it would severely reduce the luminance (brightness) of the projection screens, to a level far too low for comfortable viewing.

As has been mentioned, the scale of the grainy appearance is considerably larger than that of the actual grain in the material. This fact is illustrated in Fig. 2.1. which shows part of a microphotometer trace across a ground glass screen obtained by using a scanning spot 100μ square and illuminating and collecting beams of N.A. 0.006; the standard deviation of the fluctations in transmittance is 23% and it can be seen that the scale of the irregularities is such that detail several hundred microns across would be obscured although the glass was "smoothed" (i.e. ground with the finest grade of emery as the last stage before

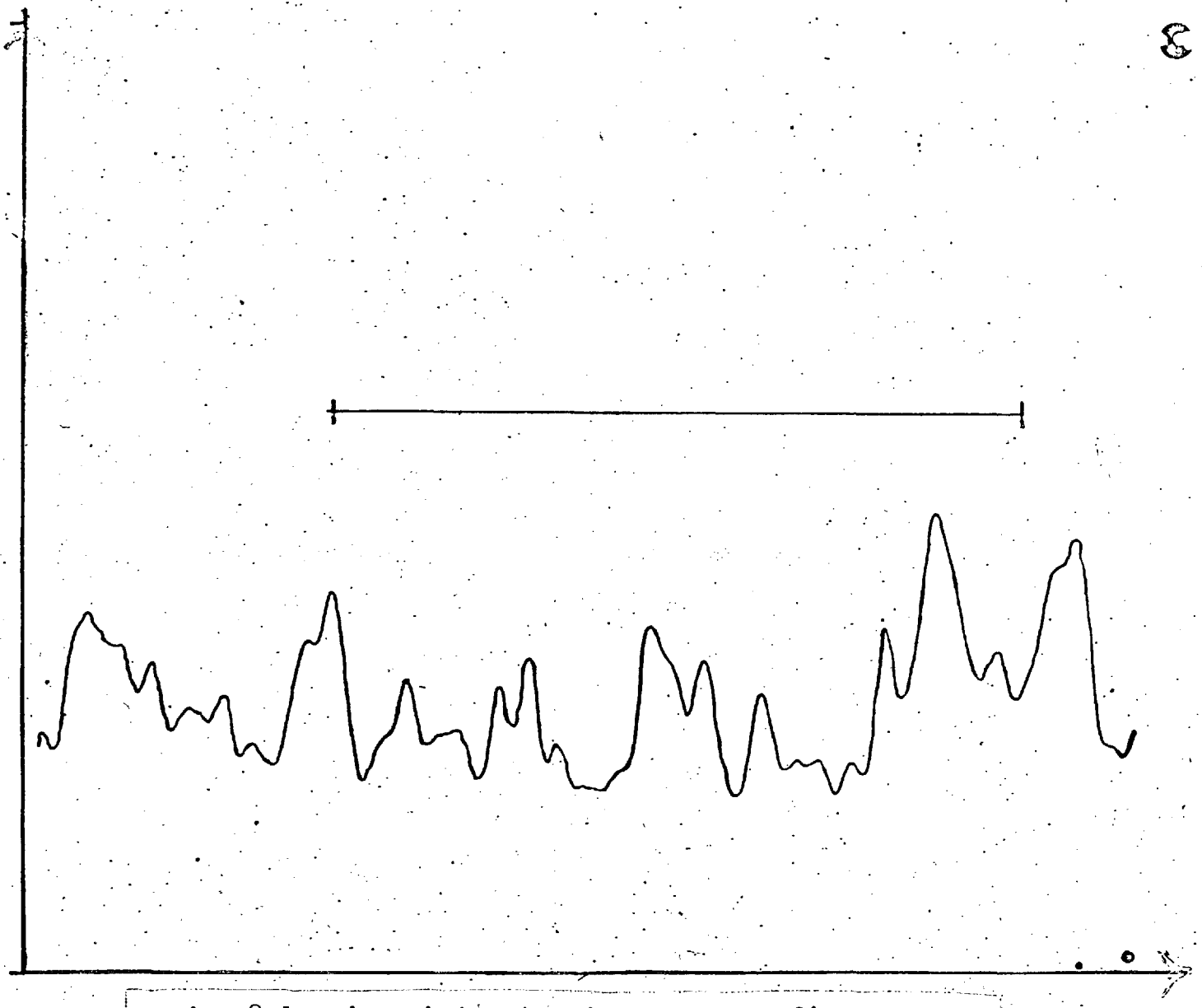


Fig. 2.1. Microphotometer trace across fine ground-glass screen. Illuminating and collecting aperture both f/80, scanning spot 100 μ square. The horizontal line at the top corresponds to 1 mm on the ground-glass screen.

polishing) amd the grain size of the emery was only about 10μ. The magnitude of the effect increases with decreasing numerical aperture i.e. increasing coherence of the illuminating and collecting beams, the values being chosen here to correspond approximately to conditions in projection microscopy.

This difficulty of graininess with small numerical aperture of the illuminating beam is found with all kinds of screens to a greater or less extent and it is probably unavoidable. A screen must have irregularities several microns in size if it is to scatter at all and these must be arranged in a random manner so that the screen does not simply become a two-dimensional diffraction grating; it is presumably the linear scale of the random variations in the screen structure which gives rise to the seen graininess, just as the graininess in a photographic emulsion corresponds not to individual grains of silver but to variations in grain density and clumping.

Perhaps the most striking example of this phenomenon is the so-called 'laser sparkle' produced when a scattering screen is illuminated with a laser-beam. The scale of this 'sparkle' or grainy appearance is again many times greater than that of the actual grain in the material of the screen itself, and is a result of the high degree of coherence and small divergence angle of the laser beam.

It can be seen therefore that in order that projection viewing should have a usefulness at least equal to that of direct viewing, this difficulty of grain must be circumvented and a grainless screen for projection microscopy produced. The development and construction of such a grainless screen will be dealt with in Chapter 5.

2.3. Sizing Techniques

If the image of an opaque extended object (Fig.2.2.(a)) were formed according to the laws of geometrical optics then its boundary would be sharply defined (Fig.2.2.(b)) and unambiguous measurement of the object dimensions would be possible. Diffraction however causes the edge of the primary microscopic image to display a gradation of light intensity whose exact form depends upon the nature of the object and the optical parameters of the microscope (Fig.2.2.(c)). In general, no simple relationship exists between the geometrical image of the edge and this intensity gradient. When such an image is observed visually using for example, an eyepiece, the distribution of subjective brightness seen (Fig.2.2.(d)) differs again from the intensity distribution in the image due to the non-linear response of the eye to light flux and the Mach effect associated with the changing luminance gradients in the image. If the observer attempts to assess the size of the object, the accuracy of the 'visual size' of the object so obtained will depend upon how nearly the position selected by the observer

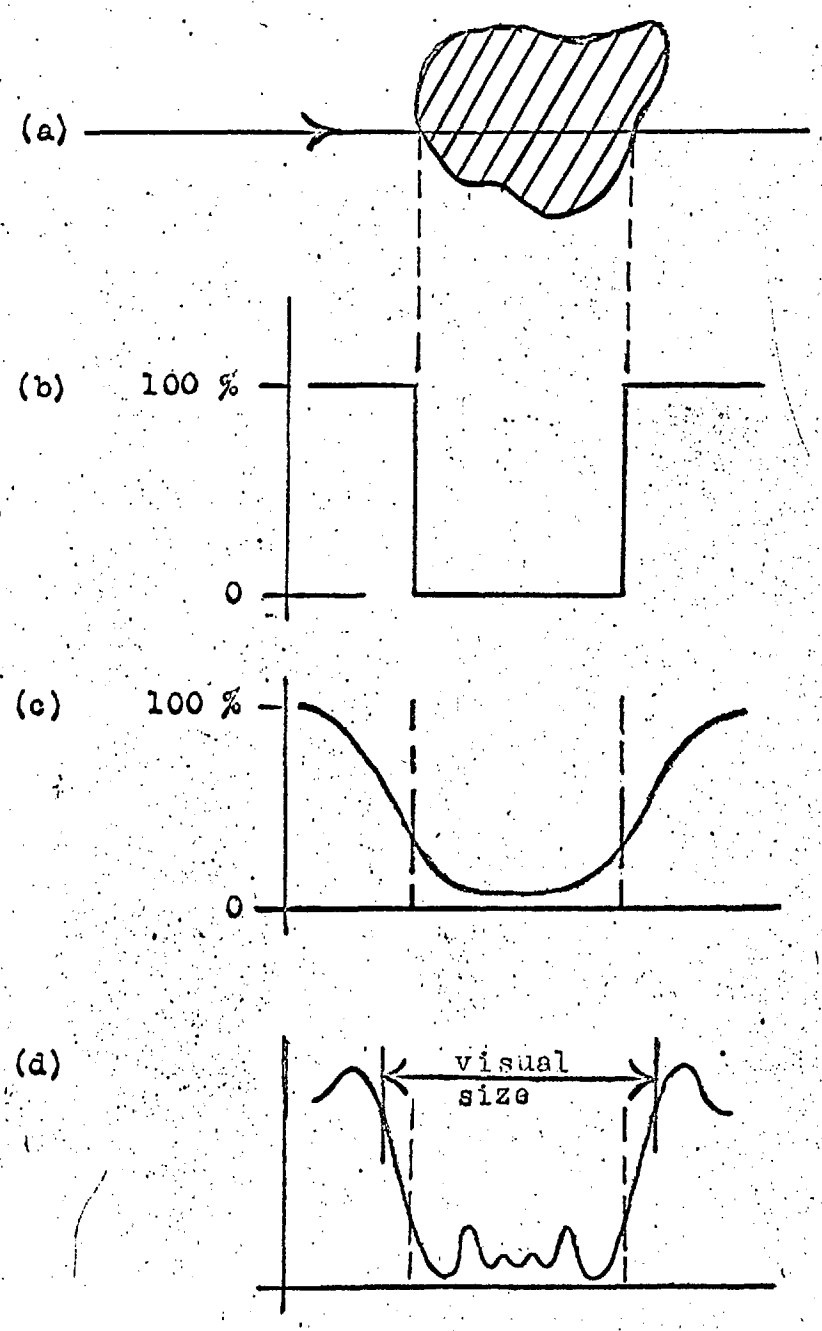


Fig.2.2. Microscope images: (a) the object; (b) light intensity across the line in (a) neglecting diffraction effects; (c) the effect of diffraction; (d) visual image showing Mach bands.

as representing the edge of the image corresponds to the geometrical image of the edge. In this section it is proposed to discuss, how the position finally chosen by the observer is influenced by the sizing technique in use, the effect of the actual form of the diffraction image, and the physiological properties of the observers visual system (i.e. the optical and visual factors) being left to the next section.

Two sizing techniques will be discussed. The first is a graticule method, namely the filar micrometer with moving crosswire, and the second is a sheared image method, that developed by Dyson (1960(a), 1961,). In the first method the observer makes his measurment by settingthe cross-wire on opposite edges of the image. According to Charman(1961, 1963(a) and 1963(b)) the most convenient and reproducible settingof thecross-wire for the visual edge in making measurements is that point where the subjective brightness of the image just differs from that of the surrounding field; and it appears that this edge criterion, though seldom explicitly formulated, is widely used in practice. Using this criterion Charman (1961, 1963(a) & (b)) has found that in general the visual size exceeds the true size of an object by an amount which for direct microscopy is of the order of half the resolution limit of the microscope objective employed, the exact value depending upon the optical and visual conditions. This difference is the systematic error

of this method. If one examines Charman's results one sees that the probable random error in a single measurment of the visual size of an object is of the order of one quarter of the resolution limit of the microscope. This random error is the setting error of this method.

In the second method the measurement is made by the observer setting alternate edges of one of the two sheared images on opposite edges of the other. The criterion for each edge to edge setting is that the two images should overlap, such that the subjective brightness at the point of overlap should be equal to the subjective brightness at the centre of each of the two images..Using this criterion Dyson (1960(a),1961) has shown theoretically that no systematic error arises in the measurement of the width of a broad, incoherently illuminated, straight edged opaque strip, of negligible thickness, the setting error being better than one tenth of the resolution limit of the objective. H e points out that where the illumination is partially coherent or where other types or sizes of object are measured, or if the object is of finite thickness as is always the case in practice, systematic sizing errors not greater than the resolution limit must be expected, although the setting precision remains high. Dyson has confirmed the value of the setting error by experiment.

The important advantages of the sheared image technique over the cross-wire method are as follows; the

estimated position of the edge is not obscured by diffraction effects caused by the filament of the eye-piece, relative movement of the object and eyepiece does not interfere with the edge to edge setting of the image since they move together, and according to Saylor (1965) only one setting is enough to determine a diameter. He claims that when using the mercury spectral line, the zero position of the eyepiece can be determined with great precision from the interference fringes that cross the field at zero displacement, the eyepiece becoming a sort of Mach-Zehnder interferometer.

The setting precison of Dyson's eyepiece has been shown to be more than double that of the cross-wire and together with all the other advantages mentioned seems to be the superior instrument. However, since one of the objects of this thesis is to compare results obtained by direct and projection methods, all that is needed is a consistent method throughout, and for reasons of instrumental convenience the crosswire method was chosen and will be described in Chapter 6, together with all the other optical details of the direct and projection microscope systems.

2.4. Optical and Visual Factors

In sizing microscopic objects the optical factors are those which affect the form of the diffraction image of the object, and the visual factors those which affect the observer's assessment of that diffraction image. Optical

factors will be considered first.

The physical light intensity distribution in the image produced by a microscope objective depends on (a) the illuminating conditions (b) the object and (c) the objective.
(a) The parameters which define theilluminating conditions are the spectral composition of the light used and its coherence, this depending in effect upon the size of the source used and upon the numerical aperture of the condenser. Hopkins and Barham (1950) have shown that providing the back aperture of the condenser is evenly illuminated and that the numerical apertures of the condenser and objective have the same values in both cases, the images formed under Köhler or critical illumination are identical. This assumes of course that the object is uniformly illuminated in both cases. While this is always so for Köhler illumination it is only true for critical illumination when the source is broad and of uniform brightness.

(b) The refractive indices and absorptions of the object and its surround as well as the three dimensional geometry of the object affect the intensity distribution in the image.

(c) The numerical aperture of the objective controls the form of the image, the focal length only affecting its lateral dimensions. Any aberrations present in the objective or defects of focus will manifest themselves by causing a change in the distribution of light in the image.

Charman (1963(b)) has shown that stopping down the condenser aperture below that of the objective tends to reduce the visual size of thin transparent objects in an opaque surround and increase it for opaque ones. His results found experimentally show that a modest reduction in condenser aperture, to a value about 0.8 that of the objective, yields visual sizes which do not differ significantly from those found in full-cone illumination and is to be recommended as improving the subjective contrast and sharpness in the image. This corresponds closely to the usual microscopist's rule that for visual work the best results are obtained when the condenser is stopped down until about 2/3 of the area of the back aperture of the objective is filled with light.

Charman has also confirmed experimentally the obvious prediction that when the objects have finite thickness the correction factors found with thin objects will no longer be applicable.

Charman's criterion for visual sizing described in the preceding section, depends upon the luminance discrimination of the eye. Therefore in considering the effect of the visual factors in size measurement, it is first necessary to see how these visual factors affect the observer's luminance discrimination.

The variation of the ratio of the just perceptible luminance difference δB of a small test field to the luminance

of its surround B with B is shown in Fig.2.3. taken from Walsh (1953,p.62.). The significant feature is that the Fechner fraction $\Delta B/B$ remains constant down to a certain luminance level below which it rises rapidly. It may therefore be expected that the same visual edge criterion under otherwise constant conditions is likely to give constant visual sizing results at values of field luminance above this certain level but that the visual size given will depend critically upon the exact value of the field luminance at lower luminance levels.

The value of the Fechner fraction also rises when the size of the test-patch is decreased. Hunt(1953) showed that the Fechner fraction tends down to a constant value providing the size of the test field is large enough, so that it may be anticipated that there will be a tendency towards a constant visual size, assuming that conditions are kept constant, if the angular subtense of the image is made large enough.

Charman(1963(a)) has confirmed experimentally the above two trends and has shown that for sizing both circular holes and opaque discs by direct microscopy, a magnification of at least 1500 X the numerical aperture of the objective and a retinal illumination of at least that corresponding to a comfortably bright image field is necessary before consistent visual sizing results are given with green light. The necessary magnification is expected to vary inversely with the illuminating wavelength.

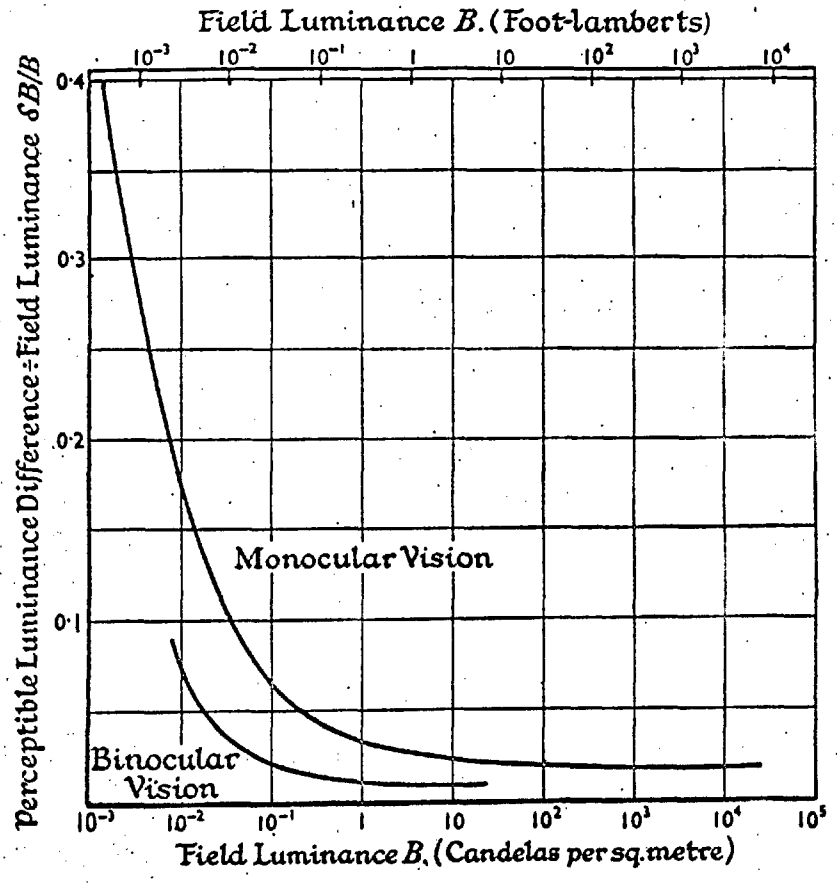


Fig.2.3. The Variation of Fechner's Fraction with Field Luminance, (after Walsh).

Charman also investigated the effect of the state of adaptation of the eye and showed that it was preferable for the observer to work in well-lit external conditions so that his eyes remained light adapted, this giving more resproducible results and reducing the fatigue involved in making measurments.

A complicating factor when attempting to compare the luminance of the image with the subjective brightness distribution seen, is the occurrence of the Mach effect when two uniform fields of differing luminance separated by a graded field are observed. This effect, which has been investigated by various authors (see, for example, the review by Fiorentini (1961) in connection with the visual perception of contours) consists essentially of the subjective perception of a bright band between the brighter luminance field and the graded field and a dark band between the lower luminance field and the graded field. Further bands may also be seen. A typical example of the difference in form between the subjective brightness and luminance distributions is shown in Figs.2.2.(c) and (d) for the image of an opaque object, although the exact brightness distribution seen varies considerably with different observers and viewing conditions.

The potential importance of the Mach effect in the particular case of visual microscopy is easily understood. Diffraction and aberration cause sharp edges of areas of different but uniform transmittances to be imaged as zones

of finite width with varying luminance between areas of different but uniform luminance; the precise distributions of luminance in these zones of the image depend upon the optical conditions in each case. These luminance distributions resemble that shown in Fig.2.2.(c). When such a distribution is viewed with, for example, an eyepiece the perceived distribution of subjective brightness will not correspond in any simple way to the luminance distribution. In general the Mach effect results in a subjective sharpening of the edge gradients (see Figs.2.2.(c) and (d)); bright and dark bands may be seen if the luminance gradients across the edge change rapidly enough. From the point of view of the microscopist, such subjective enhancement of the edge sharpness may be beneficial; it may aid the detection and recognition of detail in the image. It will moreover, improve the precision (random error), though not the accuracy (systematic error) of measurements of object size made with the microscope.

Recent investigations into the Mach effect include those of Bekesy (1960), Lowry and DePalma(1961), Fry (1963), Ratliff, Hartline and Miller (1963) and Merimont (1963), while observations into its effect on microscope images in direct microscopy have been made by Smith (1960) Charman (1961) Watrasiewicz (1963,1965(a) and 1966) and Charman and Watrasiewicz (1964).

The effect of some of the above mentioned optical and visual factors on size determination by direct and projection

microscopy will be considered experimentally in Chapters 7 and 8. Therefore it is first necessary in the next chapter to describe the preparation of suitable test objects for this purpose.

CHAPTER 3.

TEST OBJECT PREPARATION

3.1. Introduction

From the preceding chapter it is clear that the first requirement in any investigation into the factors affecting size determination is for objects of known size and shape to be available as standard test objects. These should be of as simple a form as possible, that is they should be thin sharp-edged and of uniform transmission across their area. The most useful type of object is one where either the object itself or its surround is completely opaque.

If experimental results are to be compared with the predicitions of diffraction theory, then the test objects must have forms corresponding to those for which approximate theoretical results are readily available (for example see Francon (1961(b) p.33 et seq.), that is they should either have straight edges or be circular in shape. Objects with straight edges provide difficulties because the form oftheir image may also depend upon the polarisation of the illuminating light. Circular objects however are free from these diffuclties since they have circular symmetry. A further advantage is that the production of circular apertures in an opaque film and opaque circular discs have been extensively investigated by Slater (1958, 1960) and Charman(1961, 1962) respectively. Since many of the specimens which have

to be sized in practice appear dark in a bright field (in particular this being the case with samples of dust extracted from mine air, of which the National Coal Board counts and sizes many thousands, the present work having been connected with this programme, it was felt that opaque discs would prove the more useful type of test object, and they were therefore chosen for this investigation.

As prepared, the opaque discs have the same radii as a series of mono-disperse polystyrene spheres, manufactured by the Dow Chemical Company which have already found wide use as sub-standards in electron microscopy. Dow's prepared this range of spheres by a carefully controlled emulsion polymerisation technique, and a statistical analysis of each batch of latex has been made by Bradford and Vanderhoff (1955) using an electron microscope. The diameters of the batches of spheres as determined from their measurments, are given in Table 3 (1). S is the standard deviation from the mean $\bar{X}$ of n measurements.

The two starred sizes are more recent additions to the range and do not appear in the original paper.

By courtesy of the Dow Chemical Company, 25ml samples of the above latexes were obtained and from these opaque disc test objects were prepared by the method to be described.

3.2. The Production of Opaque Discs of Known Diameters

The technique used to produce disc test objects from

Table 3 (i) The diameters of the polystyrene spheres used in test-object preparation.

$\bar{X}$ A°	S A°	n
880	80	1164
1380	62	526
1880	76	1065
2640	60	577
3400	52	415
3650	79	438
5120	74	359
5570	108	373
8140	105	357
11720	133	315
* 18300	180	}
* 27900	1500	} Not Known

spheres of known diameter has been fully described by Charman (1961, 1962).

The procedure is a development of the technique of Slater (1958,1960) who produced aperture test objects from the same spheres, by distributing them on a clean microscope slide, and shadowing them at normal incidence with evaporated aluminium, circular holes being given on removal of the spheres. Slater found during electron microscope studies of the resultant apertures that their diameter was equal to that of the spheres to within 1.5%.

Slater's method was itself a development of the technique of Hamilton and Phelps (1956), who shadowed dust particles with evaporated aluminium at normal incidence to produce, on removal of the particles, transparent apertures in an opaque film having the same profile as the original objects.

Charman's method may be summarized as follows. A few drops of a dilute suspension of spheres (about 10^6 /cc) of the required diameter are spread on a clean microscope slide and allowed to dry. A film of rock salt followed by an opaque layer of silver is then evaporated at normal incidence onto the slide at a pressure of about 5×10^{-5}mm of mercury. The slide is then gently slid into distilled water, where the rock salt layer dissolves, so that with care, the silver film containing apertures which are accurate projections of the spheres floats off in one piece

and can be caught on a second clean microscope slide. The silver film is allowed to dry and any spheres that have not already been removed during the floating off process are gently brushed or blown off, their removal being checked with a dry 4 mm microscope objective. A few scratches made in the film at this stage facilitate later focussing on the plane of the discs. A second opaque layer of silver is next evaporated on top of the first film, this new layer adhering strongly to the glass where apertures or scratches exist in the first film. The latter adheres weakly to the slide since it was floated on. Thus when a gentle current of distilled water is allowed to fall on the slide, the first silver layer comes away easily, taking with it all the second film except for those parts which adhere directly to the glass. Accurate discs are therefore obtained with additional streaks of silver for focussing purposes.

The various stages in Charman's process of disc preparation are shown diagrammatically in Fig.3.1. while Fig.3.2., shows a typical field of 1.171μ discs, taken with a 1.28 N.A. objective. The high quality of the discs is apparent. Photoelectric scans of the intensity profiles of the discs' images show a standard deviation in half-width that is no larger than the standard deviation from the mean diameter of the original spheres. Although no electron microscope investigations have been made, it is probable that the quality of the discs approaches that found by Slater for his apertures.

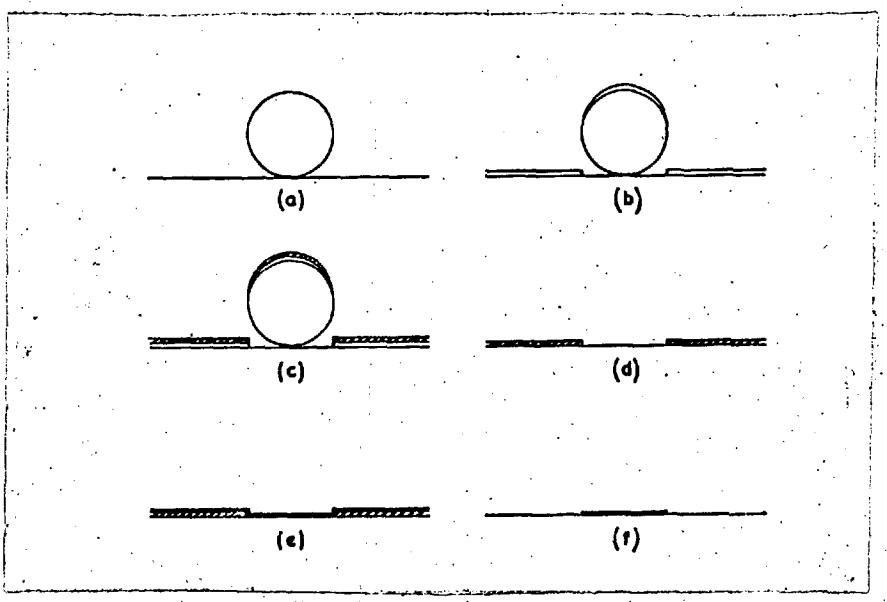


Fig. 31. Making opaque discs: (a) polystyrene sphere on microscope slide, (b) rock salt evaporated on, (c) silver evaporated on, (d) silver removed by dissolving rock salt in water and settled on fresh slide, (e) more silver evaporated on, (f) first silver layer washed off leaving opaque silver discs equal in diameter to original spheres.

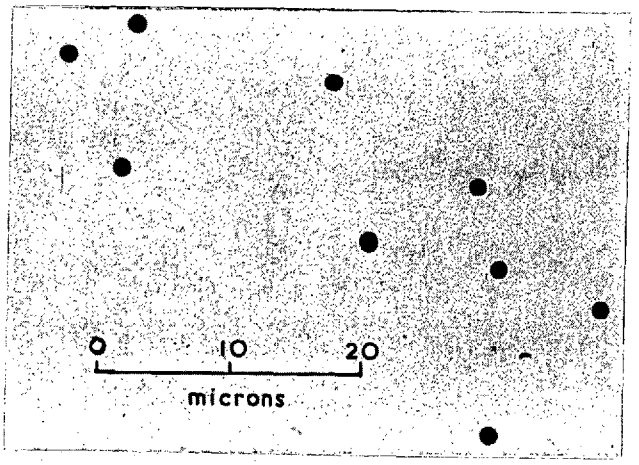


Fig.3.2.

1.171μ diameter discs.

It is clear from Fig.3.1.(c) that, if the original spheres are to be correctly profiled, then the combined thicknesses of the rock salt and silver layers must be less than the radius of the spheres. Monitoring of the rock salt thickness is a little difficult, since its refractive index (1.544) closely matches that of glass. The first silver layer must have a thickness which is equal to or greater than that of the second layer so that if the latter is to be made opaque, the first layer must be at least 800 Å thick. Therefore for correct profiling and accurate disc production the minimum possible diameter of the discs that can be prepared will depend on the thickness of the unmonitored rock salt layer.

Since in practice, a finite source-substrate distance and source size are used during the initial evaporations, the transparent apertures produced in the first silver film are not perfectly sharp-edged but are surrounded by a penumbra.

In Charman's initial experiments, the films were prepared in an Edwards 12 E coating unit, the source substrate distance being about 30 cm. The source was restricted to a disc of approximately 6 mm diameter by suitable shaping of the molybdenum boats from which the evaporations were made, so that the calculated width of the penumbra ... round the sphere profiles in the first silver

film was about 2% of the sphere radius. When the second silver evaporation was made through these apertures the diameter of the disc that was in contact with the slide was therefore approximately 1% smaller than that of the original sphere. Since this is of the same order of magnitude as the standard deviation in the diameters of the spheres themselves, this effect was not serious. There is moreover some evidence for believing that the first film may fracture half-way through the penumbrated area, with a corresponding decrease in the error in disc size. In this connection it may be noted that providing the floated-off silver film lies in good contact with the second slide, there appears to be no raggedness at the edge of the discs that would indicate irregular tearing of either of the films in spite of the comparatively rough treatment they receive. Charman using the above mentioned source substrate distance succeeded in producing discs down to a diameter of 0.557 μ. The author using Charman's method and a source-substrate distance increased to about 45 cm (thereby decreasing the penumbra effect by a factor of about two-thirds), succeeded in producing discs down to a diameter of 0.264 μ. The reason for this probably was that with the larger source-substrate distance and assuming the same amount of rock salt evaporated in each case, the unmonitored rock salt layer was reduced by a factor of somewhere between a third and a half this reduction resulting in the lowering of the minimum

possible disc diameter as discussed above.

For most purposes the fact that the objects are short cylinders rather than discs is not of great importance. Their thickness (about 0.08μ) is still less than the depth of focus of the highest aperture microscope objectives, a fact which makes them easier to use as test objects than objects of greater thickness where the plane of focus is harder to define. However these test objects certainly cannot be considered as negligibly thin and this fact will be borne in mind when considering their diffraction images in Chapter 4.

3.3. The Mounting of Test Object Slides

Before test objects can be viewed (usually with an oil immersion objective of N.A. about 1.3. for maximum resolution) a cover glass has to be mounted over them on the slide. It is obvious that there will be many advantages in using a mounting medium and not dry-mounting : (a) the resolution will be increased, since the full numerical aperture of the objective can be used, whereas with a dry slide the maximum effective N.A. is 1.0; (b) the depth of focus will be greater when using an immersion medium for the same N.A. for the focal range δl is given by the expression

$$\delta l = \pm \frac{\lambda}{8\, n \sin^2 \frac{1}{2} U} \qquad \text{---------3 (i)}$$

where λ is the wavelength of the light, U is the semi-angle of the cone of light collected by the objective and n is the

refractive index of the immersion medium ; equation 3 (i) being based on Rayleigh's limit of $\pm \lambda/4$ for the maximum mutual path difference tolerable at the image; (c) the luminance of the image will be increased according to the square of the N.A., a gain which will be of importance in projection work; (d) the air path in the dry slide introduces positive or under-corrected spherical aberration, which gives a less well-defined image; (e) the air-glass surfaces at the cover-glass and slide contribute by multiple reflections at very oblique angles to scattered light in the image, thus reducing the contrast. In addition to these optical advantages there is the practical advantage that a suitable mounting medium would provide an absolutely permanent slide. The only possible disadvantage in using a mounting medium is that transparent objects will be made almost invisible. Although this fact does not affect the present investigation it will be dealt with a little later on.

In the present investigation as only opaque test objects will be used it is necessary to find a suitable mounting medium whose refractive index is the same as that of the glass in the system and also the immersion oil used i.e. about 1.5. The obvious choice is Canada Balsam since it has a refractive index of 1.52. and is used in cementing optical components. All the test object slides were therefore mounted with Canada Balsam, thereby obtaining all the above mentioned improvements in image contrast and detail.

Turning now to the problem of transparent objects, that have a refractive index of the same order as that of glass i.e. about 1.5., as mentioned above they would be made almost invisible if a mounting medium such as Canada Balsam was used. It is therefore necessary to find a mounting medium of rather highter refractive index, say about 1.65 so that the transparent particles would show up, and so that there would be no loss of numerical aperture in the oil immersion objective. At the same time the refractive should not be too high because this would introduce spherical aberration. Leifer, Spencer and Welford (1961) have used an Aroclor resin for the above mentioned purpose in connection with sizing coal and stone dust particles for the National Coal Board, the coal being opaque and the stone which is mainly silica being transparent, with a refractive index of 1.54. The medium they used was suggested by Dr. E. Bovey of the British Scientific Instrument Research Association and was Aroclor 4465 an epoxy resin made by Monsanto Chemicals Ltd. This is a clear resin of refractive index about 1.68. Using Aroclor it was found as expected that compared with air mounting the depth of focus was increased, the field of view was brighter, there was an appreciable gain in crispness and brilliance, and the transparent stone particles could still be quite clearly distinguished, All these facts are well illustrated in Figs.3.3. and 3.4 which show coal and stone dust particles

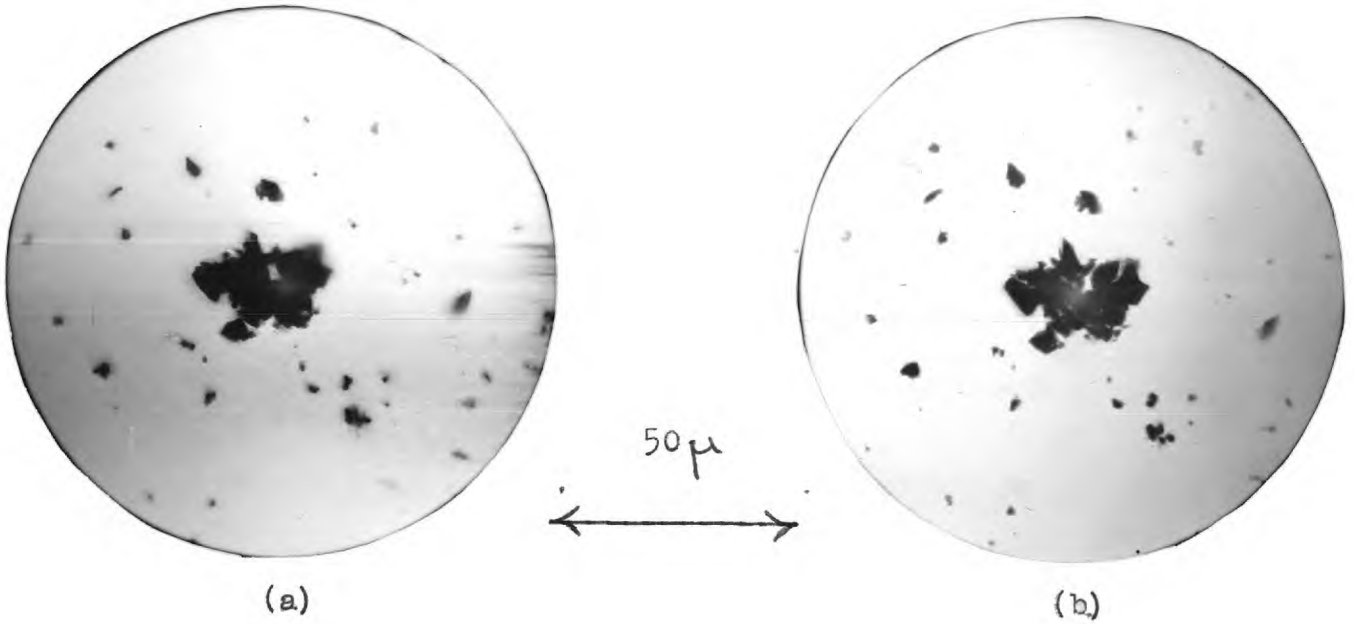


Fig.3.3. Coal dust particles mounted (a) in air and (b) in Aroclor

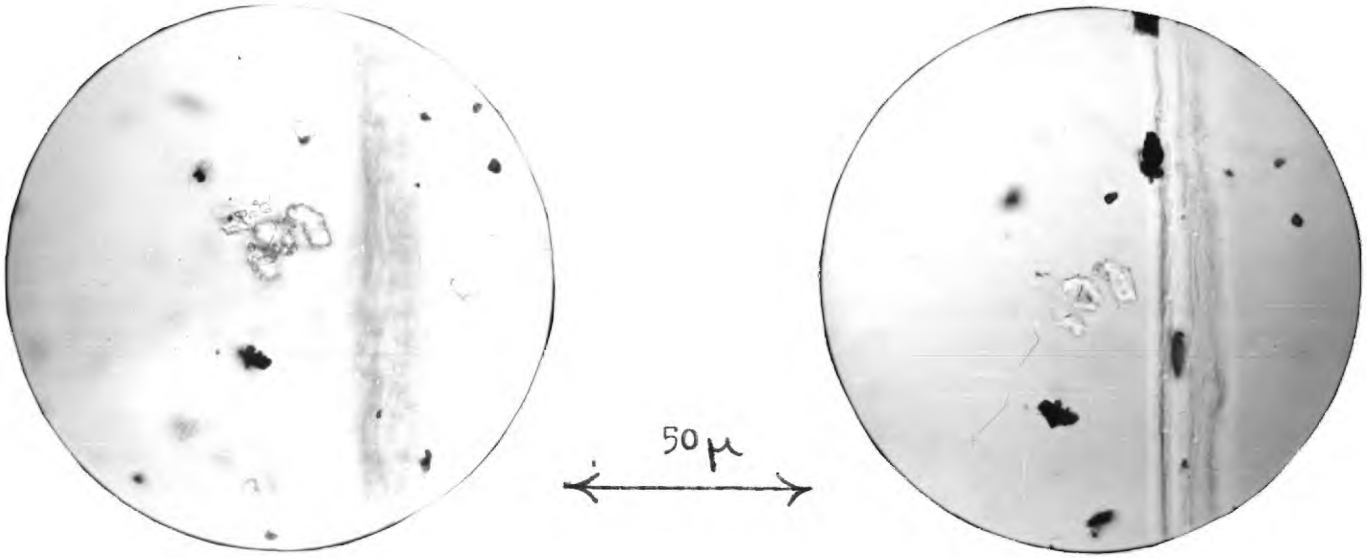


Fig. 3.4. Stone dust particles mounted (a) in air and (b) in Aroclor

respectively, mounted in air and Aroclor, the photographs having been taken by the author with a 1.3. N.A. objective. It must be admitted however, that it has not been possible to reproduce completely in the photographs the remarkable gain in clarity seen through the microscope, although the advantages of the mounting medium are quite clearly visible.

At this point it should be noted that the conclusions of Spinnel and Loveland (1960) regarding the importance of correct cover-glass thickness in reducing spherical aberration in the images of the mounted objects, were borne in mind.

Returning now to the opaque disc test objects mounted in Canada Balsam, it is obvious that before they can be used in the investigation into sizing by direct and projection methods, a knowledge of their diffraction images is necessary. These will now be considered in the next Chapter.

CHAPTER 4.

THE DIFFRACTION IMAGES OF OPAQUE DISCS

4.1 Introduction

In this chapter it is proposed to describe experiments to determine the forms of the diffraction images of the opaque discs, whose preparation and mounting were described in the previous chapter, given by a microscope objective of high numerical aperture (NA = 1.28). The diameters of the opaque discs were 0.264, 0.365, 0.511, 0.814 and 1.171 μ, and they were of the order of 0.08 μ thick. The apparatus used for the determination was the photoelectric micrometer microscope of Charman (1963 (c)). However before considering these experimental results, it is first necessary to consider the theoretical results available for the opaque disc type of object.

4.2. Theoretical Results.

All the theoretical investigations that have been made so far concerning opaque discs have been only for those having negligible thickness. The calculations involved are extremely laborious and the theory as applied is small angle diffraction theory, this only being strictly applicable, where the angles of diffraction do not exceed 0.1 radians the physical theory also being simplified by the assumption that the light disturbance can be represented by a single

scalar variable. Since in high-resolution microscopy the objective and consenser may take in cones of light of semi-angles greater than $\pi/3$ radians, the theoretical results so far produced can only be regarded as being of limited usefulness. However it does appear that there is approximate qualitative agreement between scalar theory and the results obtained with high-aperture systems.

The distribution of light intensity in the image of a single uniform opaque circular particle in a uniform transmitting surround when the object is illuminated by a narrow cone of axial illumination has been extensively investigated by Osterberg and Pride (1950). The theory as applied is the scalar diffraction theory mentioned above the objective being taken as having uniform transmission across its pupil.

Weinstein (1955) has calculated the light flux distribution in the images of incoherently illuminated circular opaque discs. Completely incoherent illumination rarely exists in microscopy however, so the results are of limited usefulness.

Osterberg and Smith (1960) have extended scalar diffraction theory to cover the case of their disc-shaped particles in a uniform surround, their solution holding for equal condenser and objective numerical apertures, although the diffraction integrals are stated for any value of condenser aperture. Smith (1960) in a following paper has

computed the energy densities for various types of disc-shaped particle using these results.

The results of Smith's calculations for opaque discs are shown in Fig. 4.1. The light flux in the image is normalised in terms of the flux transmitted by the transparent surround. The image dimensions are given in terms of 'Airy units', these being a dimensionless measure of object or image size defined as follows: let η be a dimension in the object plane, then the same dimension in Airy units is denoted by L where

$$L = \frac{(N.A.)}{0.61\lambda}\eta$$

λ being the wavelength of the illuminating light in vacuo, and N.A. obviously the numerical aperture of the objective. The quantities η and λ must be measured in the same units, then L. will be dimensionless. Image dimensions can clearly be specified in exactly the same way using the quantities appropriate to the image space. One Airy unit is the radius of the first dark ring of the Airy diffraction pattern, this of course being the usually accepted value for the resolution limit of an objective. The results plotted in Fig. 4,1. are for opaque discs having diameters 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 4.0 Airy units. The interesting features of the curves are the comparatively regular form of the image intensity distributions under these conditions and the way in which the intensity at the geometrical images of the edges

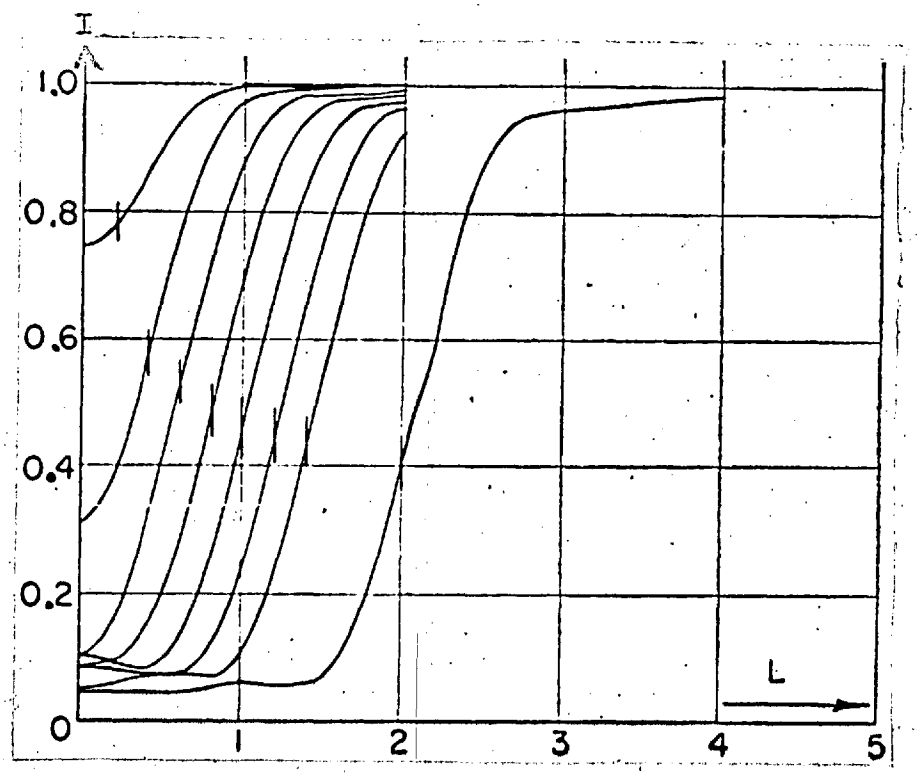


Fig. 4.1. Diffraction images of opaque discs as calculated by Smith. The short vertical line on each curve indicates the radius of the corresponding geometrical image.

of the particles, indicated in the figure by the short vertical lines, varies with the particle size.

Slansky (1960) has computed the diffraction images of opaque discs having diameters 1.04, 2.09, and 3.13 Airy units for different condonser apertures, his calculations corresponding almost exactly to thoseof Smith.

The effect of the condonser aperture on the image of an opaque disc is illustrated in Fig.4.2. taken from Francon (1961 (c) p.41). As before the intensity is normalized in terms of that obtained with the surround alone, and the diameter of the disc is approximately 0.6 Airy units. The three curves shown are for :-

(1) Axial coherent illumination, where the condenser aperture approaches zero.

(2) Partially coherent illumination, where the numerical apertures of the condonser and objective are equal.

(3) Incoherent illumination corresponding to a condenser of infinitely large numerical aperture.

The geometrical width of the imaged disc is shown in the figure by the distance included between the two small vertical lines above the curves, and the significant feature of these curves is their differing position with respect to this true geometrical image of the edge of the particle. It can also be seen that there is no one point common to all the curves that can be related to the true edge position. Thus it is clear that the degree of coherence of

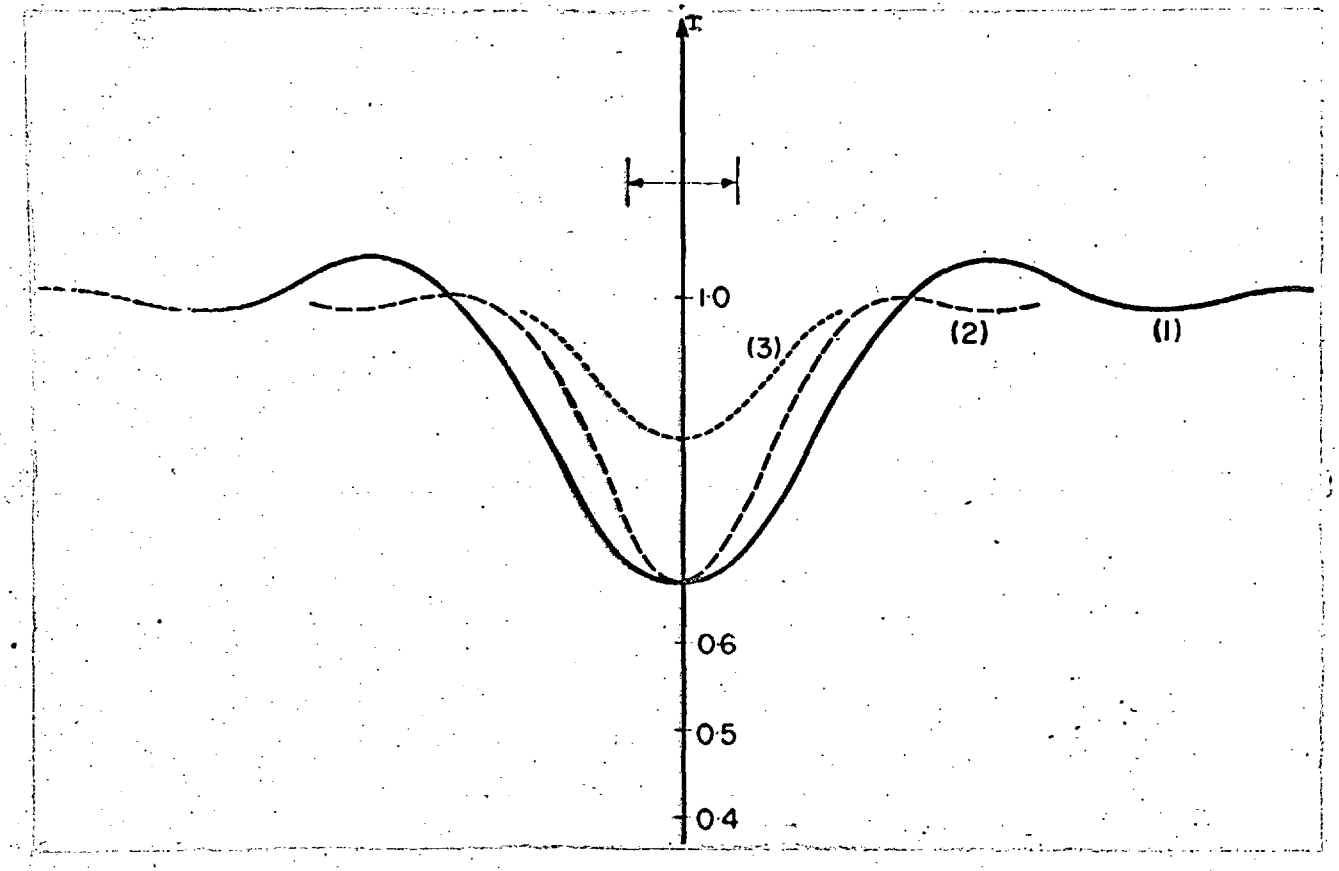


Fig. 4.2. Image of a small black disc in relation to the aperture of the condenser (after Françon): (1) coherent illumination; (2) partially coherent illumination; (3) incoherent illumination. The geometrical width of the imaged black disc (0.6 Airy units) is shown by the distance included between the two small vertical lines above the curves.

the illumination will affect any sizing process conducted with the microscope, the coherence given with an extended source generally being specified in terms of the ratio, the numerical aperture of the condenser to that of the objective, this being the 'S' value of Hopkins and Barham.

4.3 Experimental Results

Investigations into the forms of the diffraction images of opaque discs given by a microscope objective under different optical conditions have been carried out by Charman (1961, 1963(c) and 1963(d)). The apparatus he used for this purpose is shown schematically in Fig. 4.3. taken from Charman (1963 (c) p.411). Light from a tungsten ribbon filament lamp enters a modified Wadsworth monochromator whose exit slit is imaged in the plane of the object by an achromatic oil immersion type microscope condenser. The geometry of the apparatus is such that the full aperture of the condenser is evenly filled with light under these conditions. The object is thus illuminated by monochromatic light of variable wavelength, the aperture of the illuminating cone being adjusted by the usual iris diaphragm placed in approximately the back focal plane of the condenser. This system is effectively equivalent to critical illumination with the uniformly illuminated entrance slit of the monochromator as the source. The optical parameters of the apparatus are such that the width of the image of this slit in the plane of the object is fairly large (15 μ) compared with the

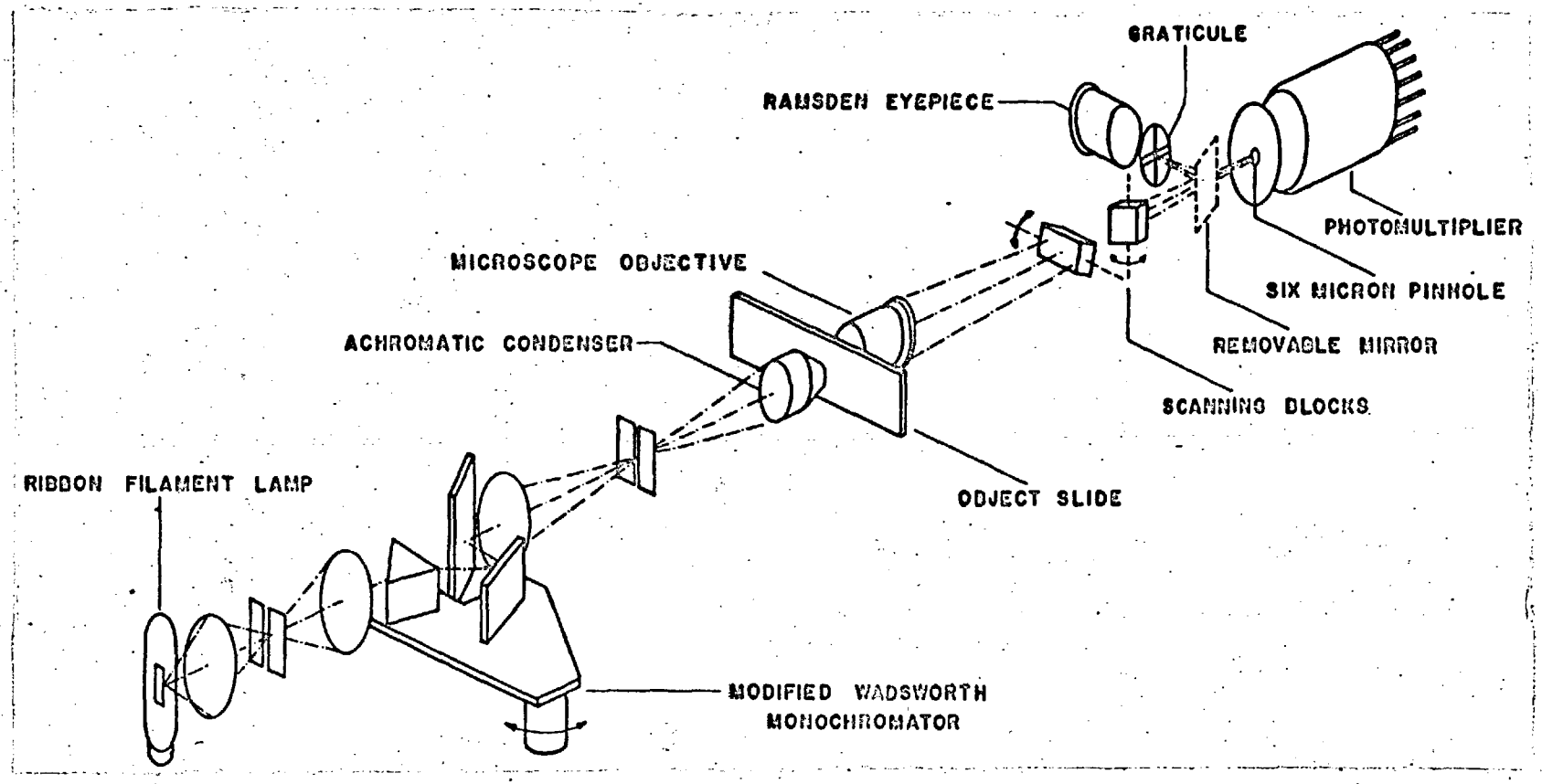


Fig. 4.3. Optical layout of Charman's photoelectric micrometer microscope.

diameters of the test objects in use, while the illumination is constant to within ± 1.0% over the central 10 μ of the slit image. Since the entrance pupil of the condenser is also evenly filled with light, the images given are identical to those obtained with Köhler illumination. The distribution of light in the primary image formed by the microscope objective is found by scanning it across a small pinhole (6 μ in diameter) placed in the imaging plane, the output of the photomultiplier being proportional to the light flux passing through the pinhole. The scanning movement is produced by rotating either of the two 1 cm thick, parallel sided glass scanning blocks about a perpendicular to the optical axis, the axes of rotation of the two blocks being at right angles to one another. The rotation is controlled through a system of levers by micrometers, a one inch traverse of one of the micrometer heads producing an image movement equivalent to about 8 μ in the object space with the high- resolution objective used in the experiments, so that image movements of 0.04 μ could easily be obtained under these conditions. The removable mirror, located on a kinematic mount, allows the image to be observed visually the planes of the pinhole and eyepiece being parfocal.

The high-resolution imaging objective was a Bock 1.8 mm apochromat having an N.A. of 1.28 and a primary magnification of 96X. Its quality was assessed by the artificial-star and Twyman-Green interferometer tests, which showed that in the

green region of the spectrum and at its optimum tubelength the axial correction of the objective was nearly perfect.

In determining the form of the diffraction image of a particular disc, the image was first focussed and centred approximately using the viewing eyepiece. On removing the viewing mirror, the image fell on the photomultiplier pinhole and could be accurately centred using the two scanning blocks. A diameter of the image was then moved in steps of 0.04 μ across the pinhole, the output of the photomultiplier as registered by a sensitive microammeter being recorded at each point. These signals were then expressed as percentage transmittances by comparison with the signal given when the object field was completely transparent. The transmittance profile of the image could thus be plotted. Dimensions in the image plane were determined by the use of stage micrometers several of which were used to give a calibration accurate to $\pm$ 0.5%.

The transmittance profiles of the images of opaque discs of 1.171 μ (4.64 Airy Units) diameter were measured with the N.A. of the objective at 1.28 and the wavelength and bandwidth of the light at 0.53 and 0.01 μ respectively. The ratio (s) of the N.A. of the condenser to that of the objective was altered by means of the condenser iris, the numerical apertures being measured with the specially designed apertometer of Spencer and Welford (1961). Charman was therefore able to determine experimentally the influence of the condenser aperture on the image of an opaque disc,

thus confirming qualitatively the changes between curves (1) and (2) in Fig.4.2. which show the theoretical transmission profiles of the image of a 0.6 Airy units diameter opaque disc for S values of 0 and 1.0 respectively.

Charman also determined the image transmittance profiles of a series of opaque discs with the condenser and objective N.A.'s both equal to 1.28, the wavelength and bandwidth of the light being $\lambda = 0.53\mu, \delta\lambda = 0.01\mu$. In his initial experiments Charman (1961) determined these profiles for discs down to a diameter of 0.557μ. The author continuing Charman's experiments was able to determine profiles for discs down to a diameter of 0.264μ. The complete size range for the author's experiments being 0.264, 0.365, 0.511, 0.814 and 1.171μ corresponding to 1.05, 1.45, 2.02, 3.22 and 4.64 Airy units respectively for the above mentioned optical conditions. Thus using Charman's apparatus and procedure as described and under the same optical conditions just previously referred to, eight measurements were made on different opaque discs in each size group, Table 4 (1) summarizing the results found. $\bar{T}$ is the mean central transmittance and $\bar{Y}$ is the width at half the central transmittance (image half-width), their standard deviations being S' and S" respectively. $\bar{X}$ and S are the mean and standard deviation of the diameters of the spheres from which the test objects were produced, corresponding to those of the discs themselves.

The complete radial image profiles are shown in Fig.

Table 4 (i)

The images of opaque discs in full-cone illumination

$\bar{X}$ μ	S μ	$\bar{T}$%	S'%	$\bar{Y}$ μ	S" μ
0.264	0.0060	36.5	0.8	0.340	0.013
0.365	0.0079	27.9	1.3	0.440	0.012
0.511	0.0074	28.2	1.8	0.535	0.015
0.814	0.0105	21.5	0.9	0.895	0.032
1.171	0.0133	18.4	1.1	1.210	0.077

4.4. The regular form of the images under these conditions is clearly apparent. Also shown in Fig. 4.4. are points on the curves (illustrated by short vertical lines) corresponding to the positions of the geometrical images and the image halfwidths, the former corresponding to the lower set of lines.

The size range of the opaque discs used as test objects during the experiments (1.05 to 4.64 Airy units) overlaps well with the range (0.4 to 4.0 Airy units) of Smith's theoretical calculations, which were discussed in the previous section. The theoretical and experimental results for $s = 1$ are compared in Fig. 4.5 which shows the variations in image halfwidth with the diameter of the opaque disc, the broken line being drawn at 45° to either axis.

Although for the reasons outlined in the discussions of the limitations of scalar diffraction theory, exact agreement between the theoretical and experimental results cannot be expected it is evident that there is fairly close agreement between the two sets of results within the limits of experimental error as shown in Fig. 4.5. Deviation from the theoretical results outside the limits of experimental error only occurs at the lower end of the size range (about 1.5 Airy units), where one would expect the thickness of the test objects (0.08 μ corresponding to 0.3 Airy units) to influence the experimental results in the direction obtained.

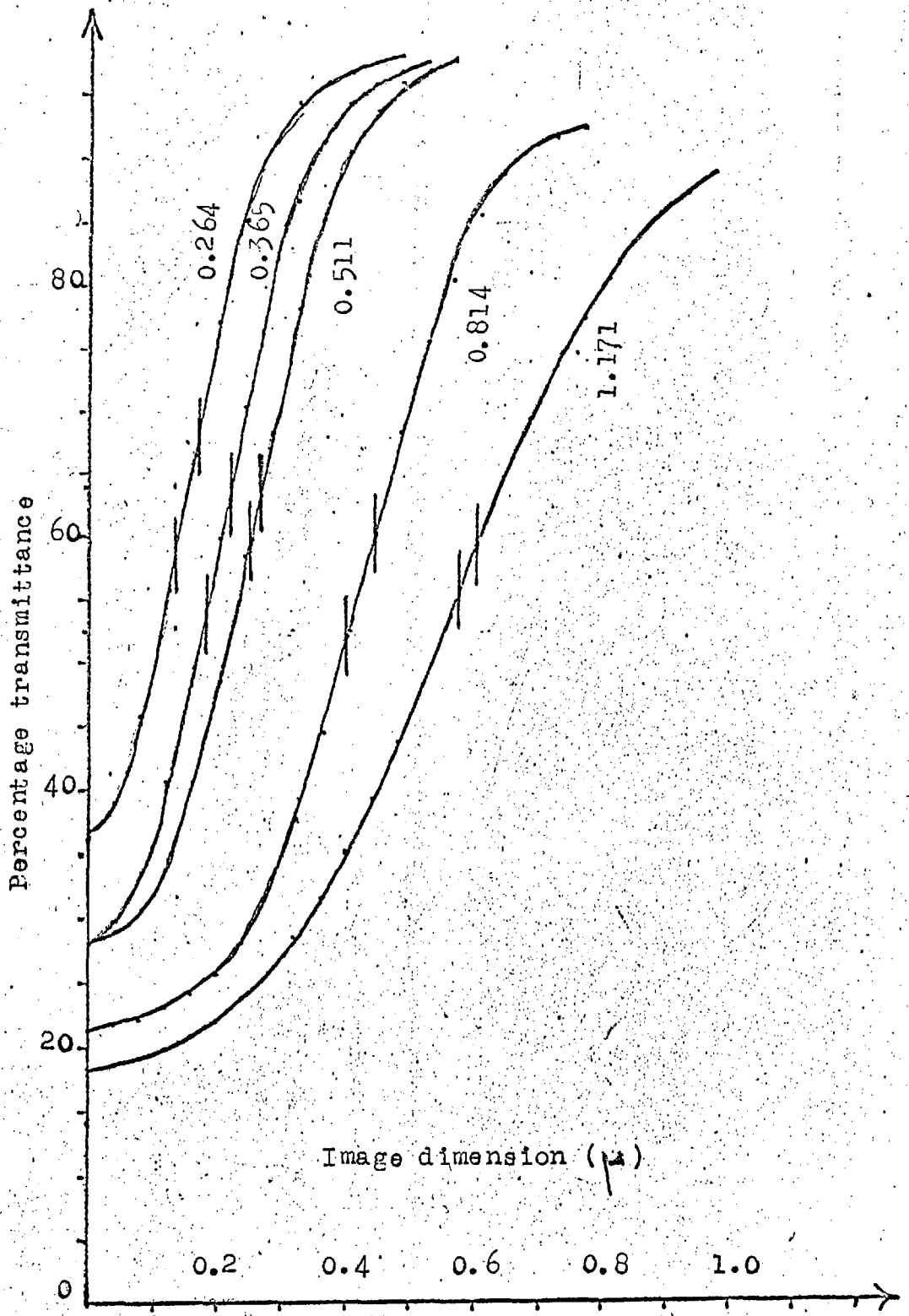


Fig. 4.4. The images in full-cone illumination ($S = 1$) of opaque discs having the diameters indicated. Condenser and objective N.A.'s = 1.28, $\lambda = 0.53\mu$, $\delta\lambda = 0.01\mu$. The upper and lower sets of short vertical lines on the curves correspond to the positions of the image halfwidths and the geometrical images respectively.

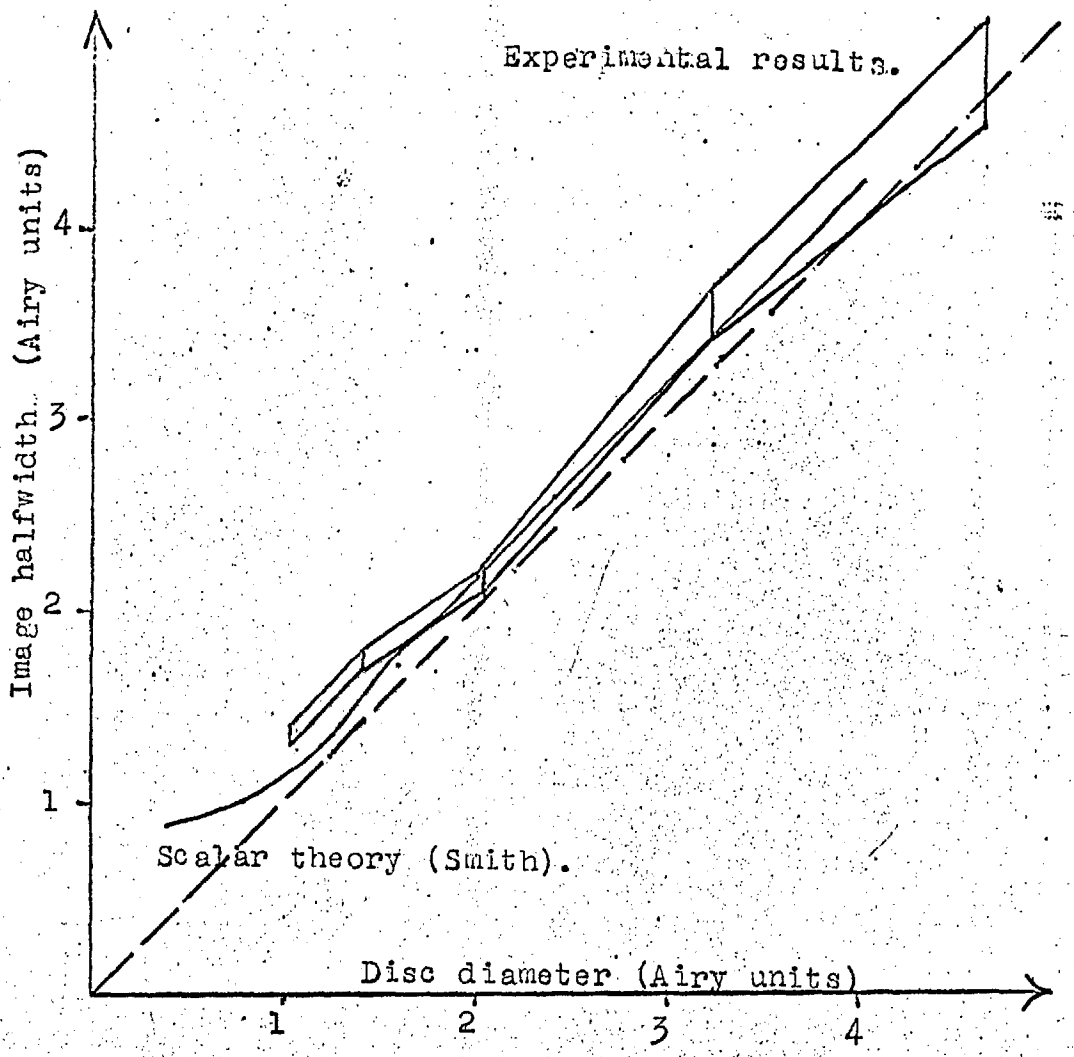


Fig. 4.5. Comparison between the experimental results and those of scalar theory for the images of opaque discs in full-cone illumination: Variation in image half-width with disc diameter.

CHAPTER 5

THE DEVELOPMENT AND CONSTRUCTION OF A GRAINLESS SCREEN FOR PROJECTION MICROSCOPY

5.1. Introduction

In Chapter 2 it was decided that in order to carry out an investigation into visual size determination by direct and projection microscopy, it was first necessary to have a projection screen which did not exhibit the property of graininess. The purpose of this chapter therefore will be to describe the development and construction of such a screen, the phenomenon of graininess itself having been adequately described in Chapter 2.

The basic principle behind grainless projection is simple. It is to produce a screen whose graininess and sparkle continually change at a rate fast enough to be smoothed out by the persistence of vision in the eye, thus giving a perfectly grainless smooth screen. All the screens described in this chapter make use of this principle and will now be dealt with in their chronological order of development.

5.2. Single Screens

The first proposal for a grainless screen was made by Mason (1947). He suggested that in order to achieve the necessary variation in graininess the projection screen should be moved rather rapidly in its own plane. Mason's

idea was developed by Lau and Schalge (1958) who produced a ground-glass back-projection screen, which was rotated in its own plane, this idea also being referred to by Lau and Reinitz (1959).

It was therefore decided to produce a screen using the above mentioned ideas. The first was in the form of a disc-shaped ground glass back-projection screen rotating in its own plane. The image was projected on to the outer portion of the screen, and when the disc was set rotating an improvement in the definition of the image was obtained. However the steady movement of the screen through the field of view was always noticed and this was distracting, but worse still was the fact that large-scale variations in scattering over the screen showed up as a flicker with the period of rotation and could only be eliminated by using a speed of rotation above about 30 revolutions per second, a rather high speed for a thin disc of glass.

An alternative way of rapidly moving a single screen is to vibrate it in its own plane at the A.C.-power-line frequency of 50 cycles/sec. This was tried with an amplitude that could be varied up to about 1mm, but no grainless effect was obtained.

5.3. Double Screens

An improved method of achieving the grainless effect was suggested by MacAdam and Taylor (1947) for use in engineering gauge projection. It consists of two ground

glass screens placed with their ground surfaces almost in contact with one moving slowly, its own plane relative to the other. This idea was also referred to by Habell and Cox (1948).

Using the above -mentioned system it was found that even with quite slow relative speeds (of the order of 1mm/sec), because there are two screens, the sparkle and graininess vary at a sufficient speed to be smoothed out completely by the persistence of vision in the eye, thus giving a completely perfect grainless screen. The effect is quite startling for low-constrast objects of which the images are less than a millimetre in size on the screen such objects are almost invisible when the screens are stationary, but become brilliantly clear when the movement is started. Although the relative speed of the screens need only to be quite slow, the motion must be such that there are no stationary points or else if such a point does occur its effective duration must be less than, say $\frac{1}{25}$ sec; by effective duration is meant the time during which the relative speed is less than the minimum for which the grainless effect occurs.

Fig.5.1. shows an attachment for a microscope, constructed by Messrs. C.N. Richmond, The Orangery, Kelvedon, Essex, using the above mentioned principle. This kind of screen follows MacAdam and Taylor's original design rather closely. One ground-glass screen is stationary and the other

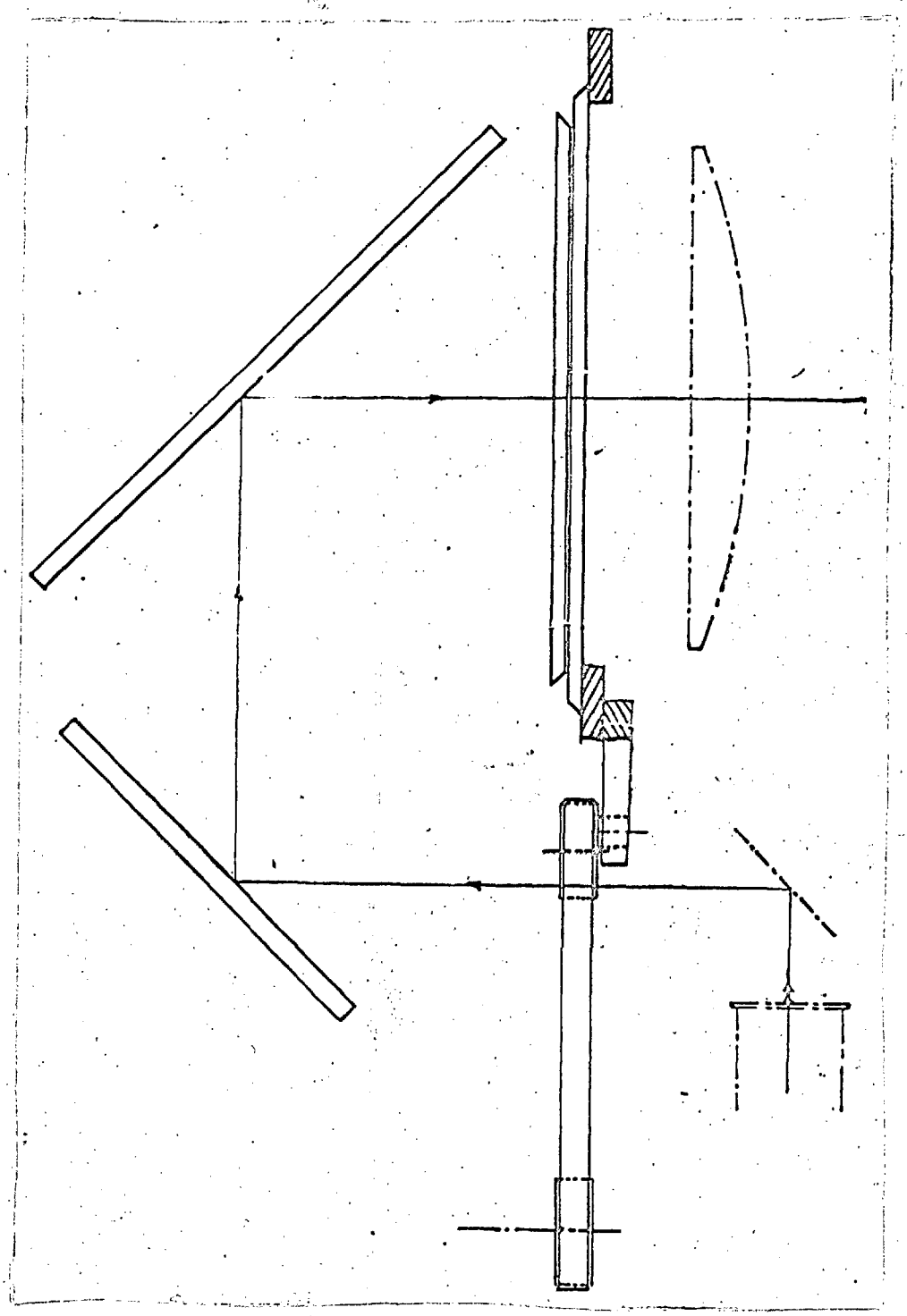


Fig. 5.1. Grainless screen. Light from microscope eyepiece (lower right) after reflection from three mirrors forms the image on two ground glass screens (upper centre) nearly in contact. The image may be viewed through a field lens (upper right) if the screens are etched for high forward transmittance. The ground-glass screen nearest the observer undergoes circular translation in its own plane at about 20 rpm from the motor drive (bottom centre). In an alternative arrangement the moving screen is oscillated in its own plane at A.C. power-line frequency.

is moved by means of an eccentric in a circular movement, the track of each point on the moving screen being a circle of about 5mm radius, which is traversed in a few seconds. The gap between the two screens is adjustable, and it is found that a separation of up to a quarter of a millimeter has no discernable effect on the quality of the image. As in the previous section an alternative way of moving one of the screens is to vibrate it at A.C.-power-line frequency with an amplitude of about 1mm. This time if a second screen is placed almost in contact with it as in the first system a grainless effect is obtained which is just as effective as that produced by the slowly moving rotating screen, this system being perhaps slightly simpler and cheaper.

Both of the above two systems can be arranged on a stand to take a microscope as in the photograph Fig.6.4. in the next chapter, which shows the slowly moving rotating screen and the rest of the projection microscope system, yet to be described.

All the systems mentioned so far in this chapter have been for back-projection. A grainless front-projection system can easily be produced simply by aluminizing one of the two screens and moving the other, as above. However although this successfully eliminates the graininess, this system is found to be far inferior to the back-projection one for the following three reasons;

(a) As has been mentioned in Chapter 1 front-projection itself is inferior to back-projection because in the former there is physical obstruction of the view by the microscope.
(b) The luminance of the front projection screen was found to be of the order of one tenth of that of the back-projection one.
(c) The grainless effect in the front-projection screen was marred by the strong specular reflection of the exit pupil of the microscope in the front polished surface of the non-aluminized screen. The effect was so strong that it could not be eliminated by the most efficient anti-reflection coating.

5.4. Etched Screens.

A three to fourfold increase in luminance is obtained with the double screen back-projection system if the ground glass screens are etched with dilute hydrofluoric acid to increase their forward transmission, as proposed by Dyson (1960 (b)). The etching causes the grinding pits of the ground glass to develop into a series of irregularly juxtaposed small concave lenses, which have the effect of concentrating the scattered beam in the forward direction, so that a very sharp polar diagram is obtained. Fig. 5.2. shows polar diagrams for light transmitted by two pairs of screens, the first pair being ground with the finest grade of emery, and the second pair being ground as above, and then etched in a 5% aqueous solution of hydrofluoric acid

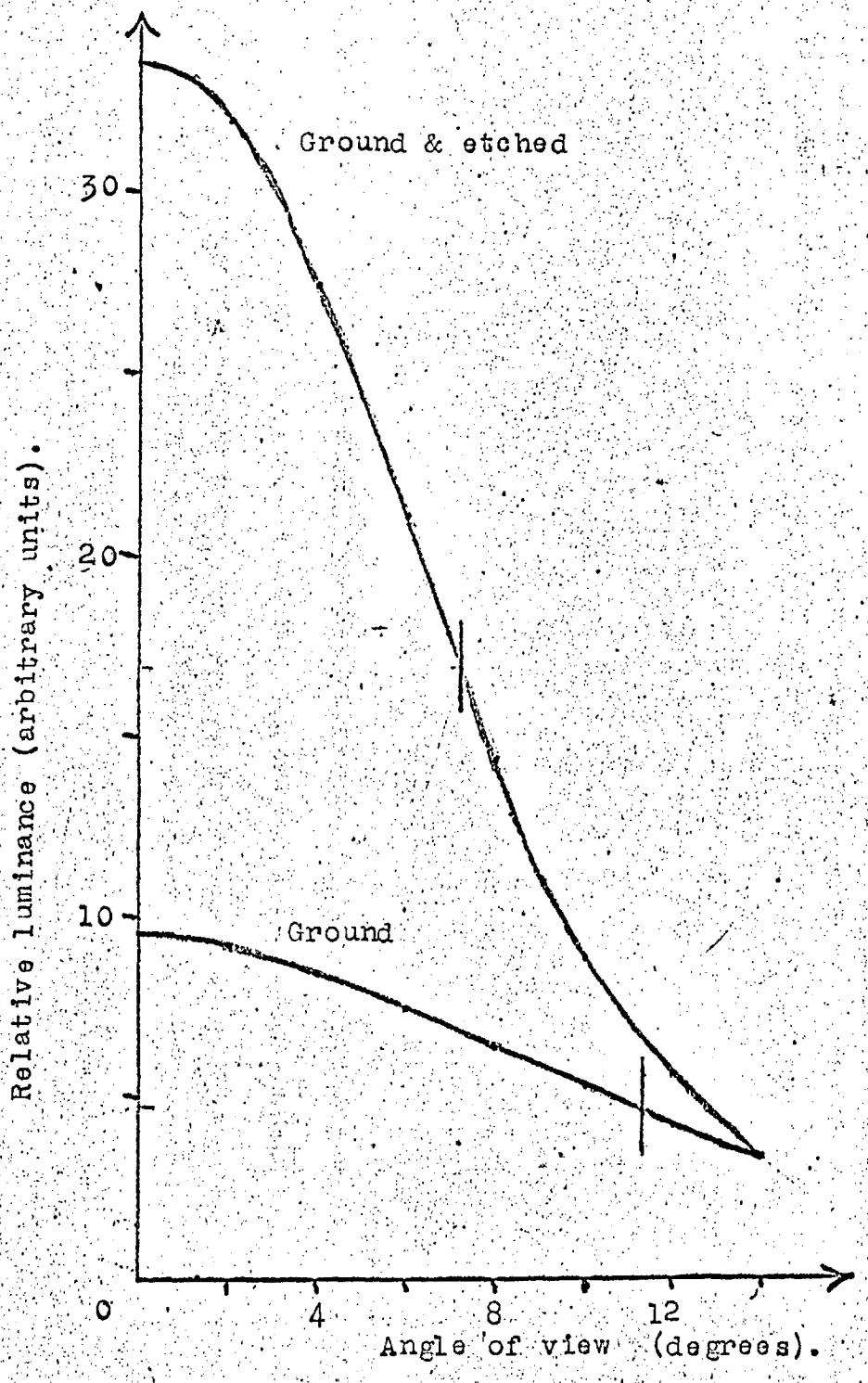


Fig. 5.2. Transmission polar diagrams of pairs of ground and etched glass screens.

for about one and half hours, the unground surface of the screens being protected with wax. From Fig. 5.2. it can be seen that the maximum luminance of the etched screens is three to four times that of the plain ground pair. However it should also be noticed that the points on the curves corresponding to half the maximum luminance (indicated by the short vertical lines), is reached in the case of the etched screens at an angular displacement from the maximum which is about two-thirs that of the same quantity in the case of the ground screens. Therefore although the etched screens will have a much higher maximum luminance when used with a microscope, the distribution of luminance over them will be less uniform. In order to overcome this it is necessary to use a field lens as indicated in Fig. 5.1. in order to collect the rather narrowly scattered beams and thus obtain a reasonably uniform field of view.

A disadvantage of the etched screens is that the etching produces a clearly visible structure on the screens which may be slightly distracting to the observer.

5.5. Summary

It was found that the best results were obtained by using:

(a) Two screens rather than one, because the grainless effect produced by the former is far superior.

(b) Back-projection rather than front-projection because of the three reasons already stated in section 5.3.

(c) Plain ground rather than ground and etched screens because of the visible structure of the latter, mentioned at the end of section 5.4.

(d) Either the slowly rotating or the rapidly oscillating double screen systems, both producing equally perfect grainless effects, although the one illustrated in this thesis is the slowly rotating system.

Therefore the grainless screen to be used as part of the projection microscope system will consist of two plain ground glass screens with their ground surfaces facing each other separated by not more than 0.25 mm, one of which is moving with a speed exceeding 1mm/sec, the whole being set up for back-projection.

At this point it should be mentioned that much of the work described in this chapter has already been described by Leifer, Spencer, Wolford and Richmond (1961), the bulk of the development work having been carried out by the author.

CHAPTER 6

OPTICAL DETAILS OF THE DIRECT AND PROJECTION MICROSCOPE SYSTEMS

6.1. Introduction

The general arrangement of the direct microscope is shown in Figs. 6.1. and 6.2. and that of the projection microscope in Figs. 6.3. and 6.4. A more detailed description of the individual components will be given below.

The components to be considered first will be those which are common to both systems, i.e. the light source and the microscopes, after which the different viewing and sizing components of the two systems will be dealt with.

6.2. The Light Source

The light source used is a 250 watt high pressure mercury arc lamp, which has a luminance of 2×10^8 candelas per square metre, when working at correct power and when used without any form of light filter. The lamp is connected across the mains supply, in series with a suitable choke, the output of the lamp being constant.

The luminance of the source can be varied only by the use of suitable neutral or wavelength filters, the wavelength filter used transmitting strongly only the green line of the mercury spectrum, of wavelength 0.546 μ.

Mercury arc lamp

Condenser lens

Iris diaphragm

Achromatic condenser

Object slide

Apochromatic objective

Scanning block

Eyepiece

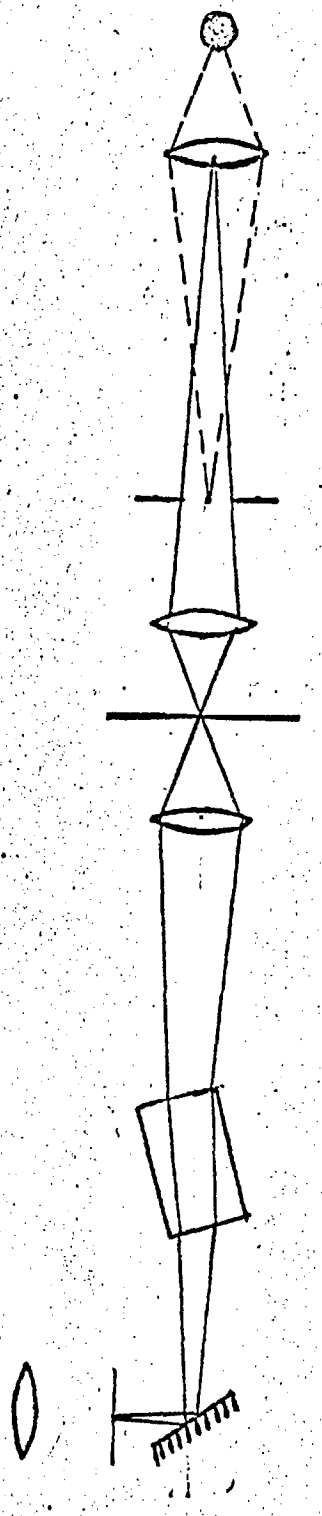


Fig.6.1. Optical layout of the direct microscope

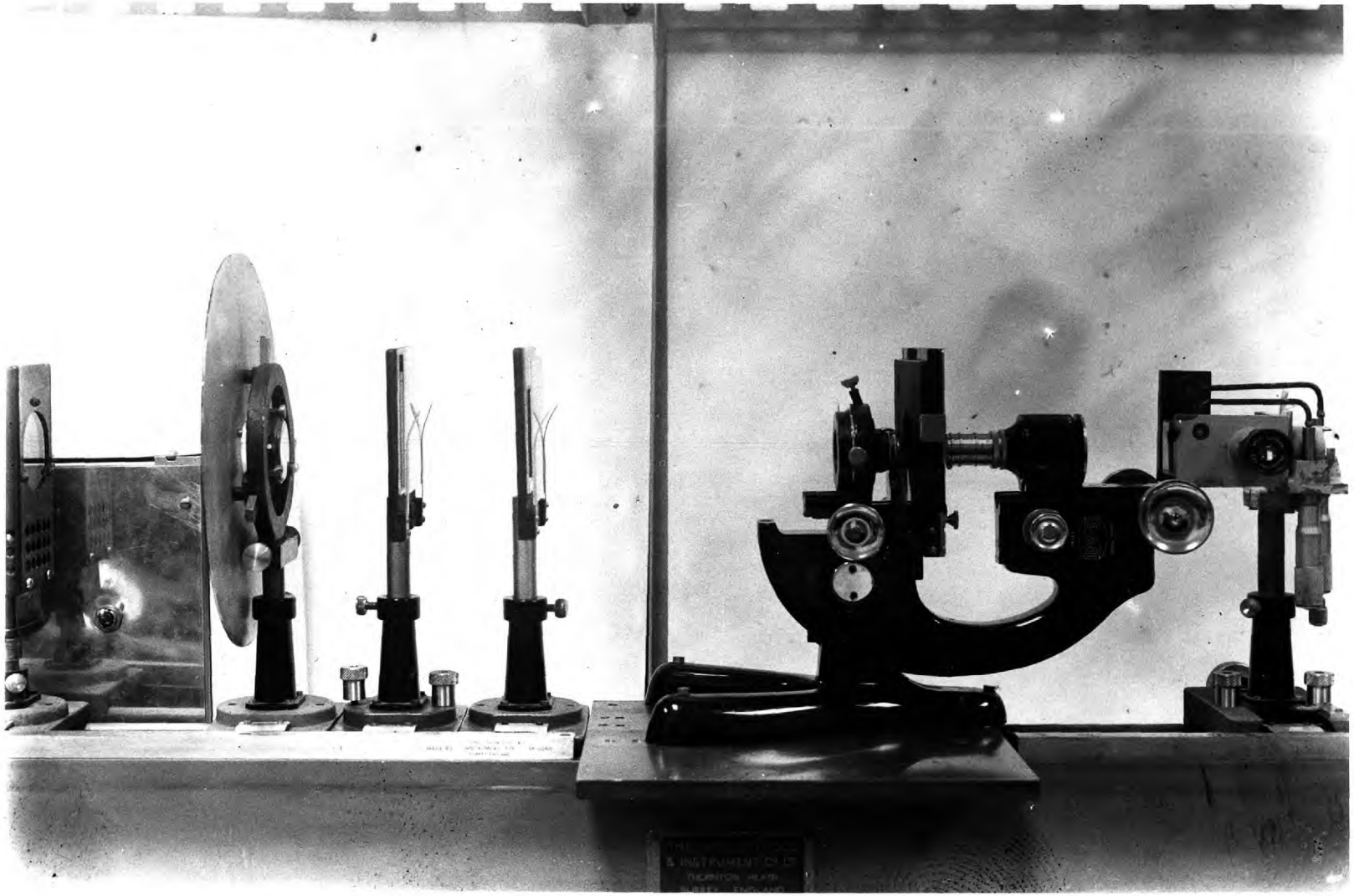


Fig.6.2. The direct microscope

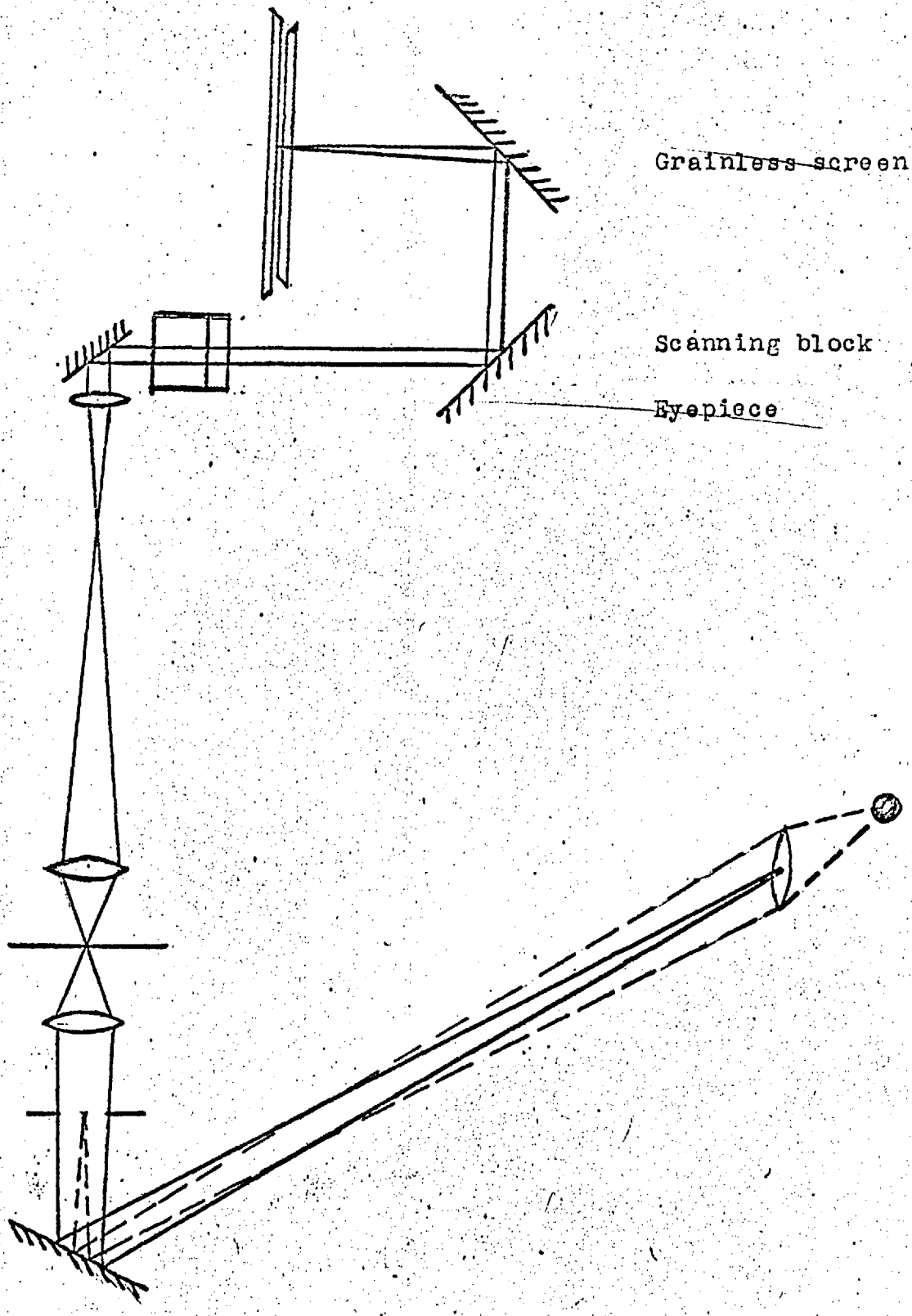


Fig. 6.3. Optical layout of the projection microscope

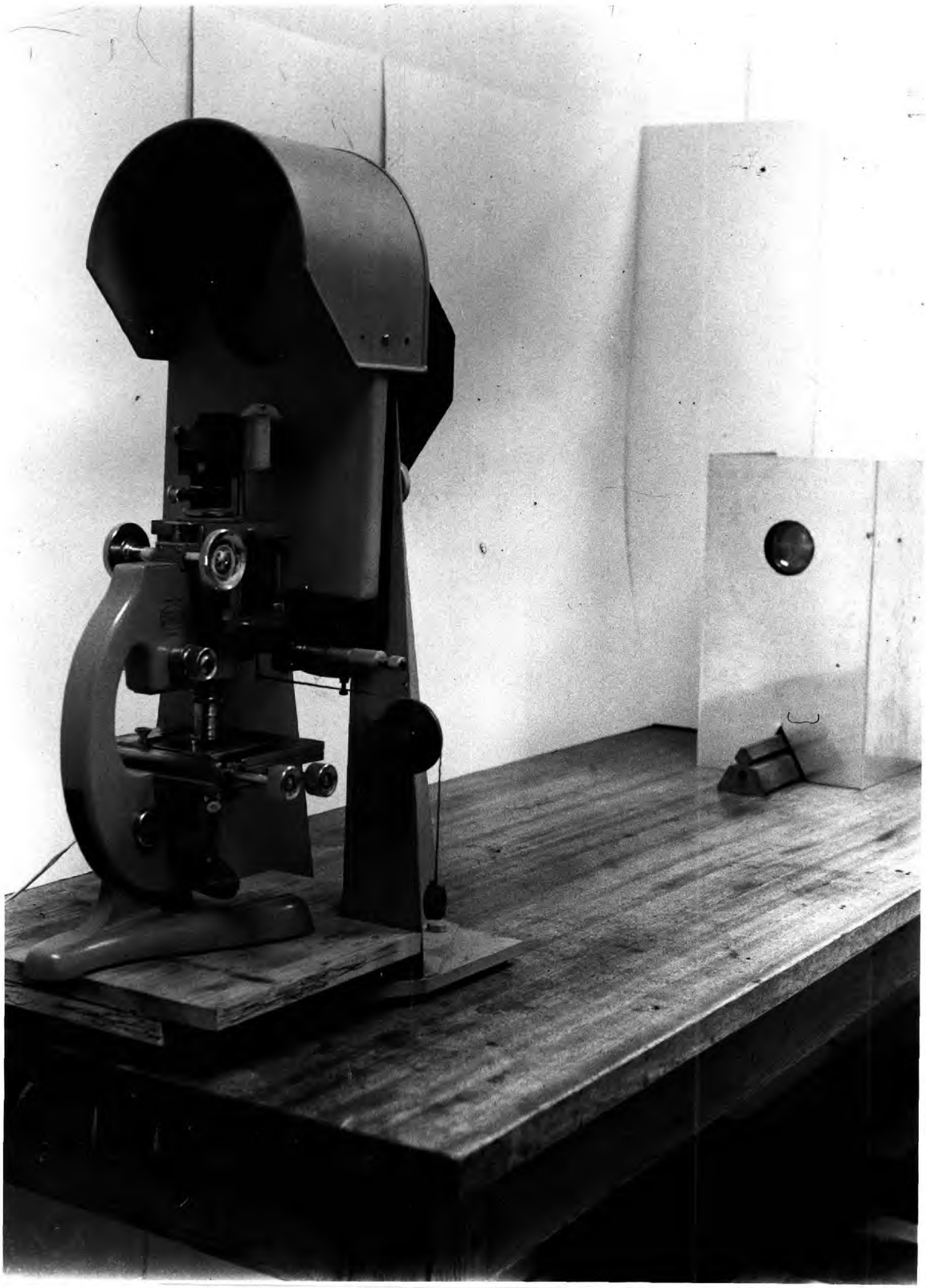


Fig.6.4. The projection microscope

6.3. The Microscopes

Both microscopes are Beck London Models, mounted horizontally in the direct system and vertically for projection. The light from the mercury lamp is focussed on to an iris diaphragm mounted in approximately the back focal plane of the condenser, the angular aperture of the beam being sufficiently large to fill the back aperture of the condenser with light.

By means of the iris diaphragm the numerical aperture of the illuminating cone can be varied from 0.2 to 1.3, the diaphragm being calibrated using the apertometer designed by Spencer and Welford (1961). The condenser is adjusted by the usual rack and pinion, the aperture of the source lens being focussed in the plane of the object.

The objective used is a Watson X90 oil-immersion apochromat with an N.A. of 1.33. Coarse focussing is again by rack and pinion, the fine focus which incorporates an anti-crash device to prevent damage to the objectives through mis-handling, being sufficiently smooth and free from backlash to ensure precise focussing with a high aperture objective.

The objective was examined by the star test, whose application has been described by Slater (1957), and which has been estimated by Welford (1960), to be capable of detecting aberrations of the order of $\lambda/20$, depending upon the type of aberration present. It consists essentially of

the examination of the in and out of focus images of circular pinholes whose diameter is less than half the resolution limit of the objective employed, these being illuminated by a condenser having an aperture at least as large as that of the objective. With perfectly corrected objectives, identical circular diffraction images are given at equal distances on either side of the best focal plane, the in focus image being an Airy diffraction pattern. The aberrations present in an objective may be deduced from the variation from these ideal conditions of the images obtained.

Since all experimental observations are made at the centre of the field, the off-axial aberrations are not important, an aberration-free field 20μ in diameter being quite adequate. The star test showed that the field of the Watson objective at full aperture was free of coma and astigmatism over a sufficiently large area, this field also being flat. Present however was a small amount of spherical aberration this being minimised when the tubelength of the microscope was adjusted to its optimum value.

6.4. The Viewing and Sizing System of the Direct Microscope

The system designed to incorporate, a rotating parallel sided glass scanning block, a mirror and eyepiece for visual sizing can be seen at the bottom of Fig.6.1. and on the extreme right of Fig.6.2. The incorporation of all these components in a single optical bench unit mounted independently from the microscope stand, ensures that there will be no unwanted movement of the image during the sizing process.

The scanning block is the one used previously by Tewari (1962) in the determination of intensity contours in the images of coal dust particles, the action of the block being illustrated in Fig. 6.5. It was originally designed to move the image in two mutually perpendicular directions. However in the present investigation only the horizontal movement is used. The block was cut with a diamond saw from Pilkington plate glass of refractive index 1.52 and mounted kinematically as illustrated in Fig. 6.5. The slots P, Q, and R in the glass block were cut with an ultra-sonic drill, and into each of these fits a small metal sphere. The sphere Q is fixed whereas the two spheres P and R are mounted on the ends of two metal levers L and M connected via pivots to two micrometer heads. The free ends of the micrometer heads make contact with two small metal spheres at the ends of the levers, contact being held by weak springs. If the micrometer connected to the lever L is moved the block rotates about a horizontal axis perpendicular to the axis of the microscope, the image thus being moved vertically. Similarly if M is moved by its micrometer the block rotates about a vertical axis perpendicular to that of the microscope horizontal movement thus being obtained.

The front-aluminized plane mirror directs the light into the eyepiece, which fits into a short length of tube of the standard eyepiece diameter attached to the basic block unit. In the back focal plane of the eyepiece there

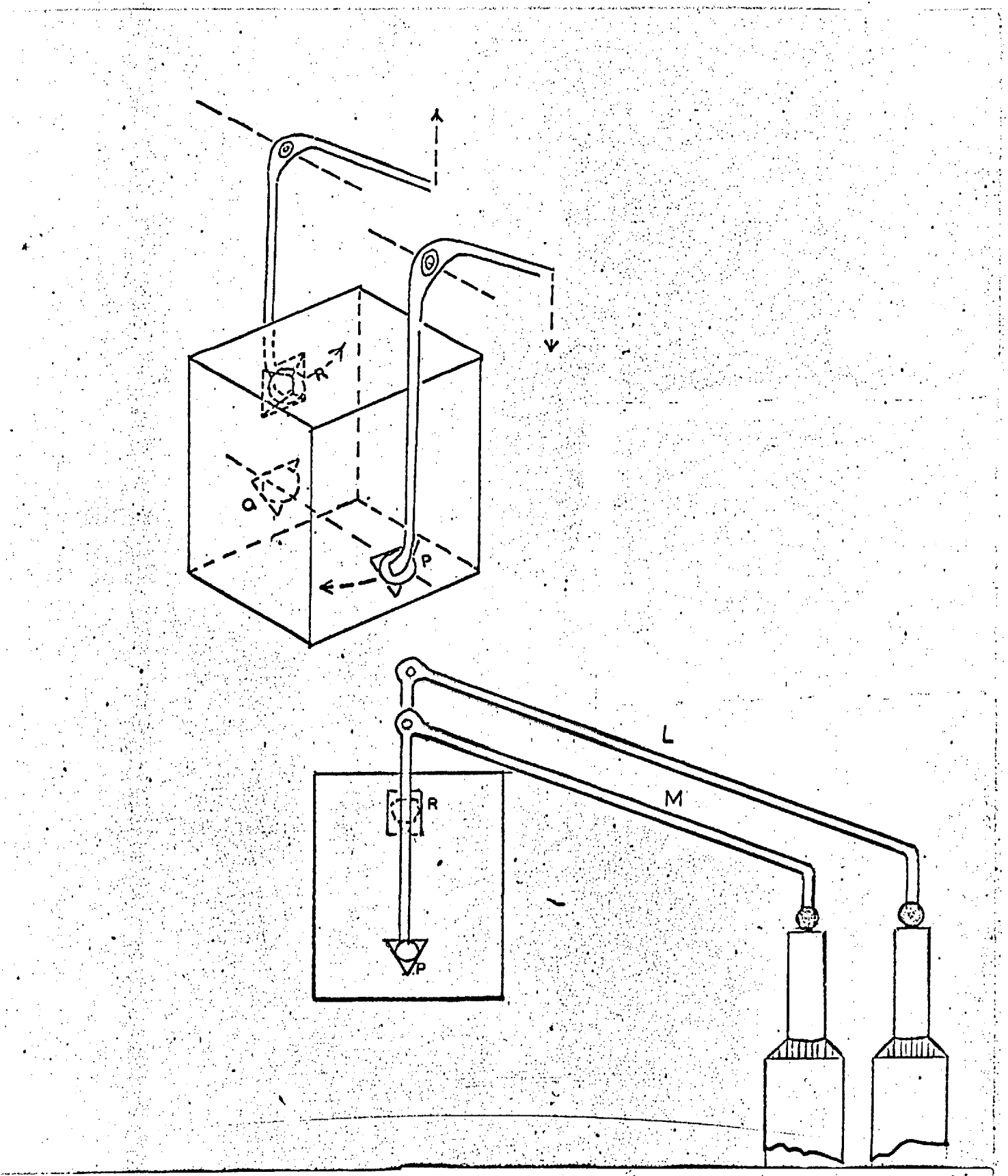


Fig.6.5. The scanning block of the direct microscope

is a vertical single line graticule, across which the image may be moved by the scanning block, acting as an optical micrometer. By setting opposite edges of the image on the line, the visual size of the object may be determined.

The scanning block must be of such dimensions and form that when rotated it can move the image in the horizontal direction by an amount corresponding to the diameter of the image of the largest size of test object. This motion must be linear to within the limits of accuracy set by the other experimental conditions and no additional degredation in the image or the linearity of the image movement should be caused by imperfections in the block itself.

The quality of the block was assessed using a Twyman-Green interferometer, and it was found that the optical thickness of the block was constant to within $\lambda/4$ over the full area of the faces. Using this block it must now be established that its rotation results in a sufficiently linear image movement.

The insertion of a parallel sided glass plate into a beam of light results in the light beam being displaced by an amount depending upon the angle of incidence upon the plate. Thus in the apparatus on rotation of the scanning block the image will be displaced laterally, the rotation of the block being controlled by the lever and micrometer head as described above. It is required that equal changes in the positions of the micrometer heads produce equal image displacements at all points of the micrometer movement.

If a flat parallel sided plate of thickness t and refractive index n, has light incident normally on one of its faces and is then rotated about its centre by an angle θ, then the emergent light is displaced by an amount d such that

$$d = \frac{t \sin\theta (n - 1)}{n} + \frac{t \sin^3 \theta (n^2 - 1)}{2n^3} \quad \ldots\ldots 6\ (1)$$

assuming θ to be sufficiently small for terms involving $\sin^4\theta$ and higher powers to be neglected.

In the microscope the lever and micrometer are arranged so that $\sin\theta$ is a linear function of the micrometer reading. The image movement will therefore be directly related to the micrometer reading providing the value of the second term of equation 6 (1) is very much smaller than that of the first, that is providing $\sin\theta$ is small.

The maximum image movement required is $\frac{1}{5}$ mm, corresponding to just over 2μ in the object space, with a primary magnification of about X90, this being adequately greater than the largest size of test object. The block is arranged so that in the middle of its horizontal rotatory movement its faces lie perpendicular to the axis of the microscope, the required maximum image movement of $\frac{1}{5}$ mm, thus being achieved by a maximum image displacement to either side of $\frac{1}{10}$ mm.

The linearity of the movement was examined using a x40 objective and a stage micrometer with a spacing of 2μ.

Thus for each movement corresponding to 2 in the object space the image was displaced 80 μ, and for each of these displacements the micrometer reading was noted in tenths of an inch over the whole range. The results are plotted in Fig. 6.6. from which it can be seen that no systematic deviation from linearity could be detected over a range of about $\frac{1}{2}$ mm to either side of the centre of the field, and that over this total range of about 1 mm. the average random deviation from linearity was less than 1%, which is less than the standard deviation of the test objects themselves. Therefore over the maximum range required (i.e. $\frac{1}{10}$ mm to either side of the centre) the linearity of the movement of the block was found to be quite adequate for the present investigations.

6.5. The Viewing System of the Projection Microscope

This is illustrated in the top of both Figs. 6.3. and 6.4. and consists of a grainless back-projection screen (described in the previous chapter) which by means of a pair of folding mirrors is arranged vertically above the microscope tube, so that all the controls are easily accessible, the screen being adjustable in height to suit different microscope tubelengths.

The luminance of a microscope projection screen can be shown theoretically to have a value B such that

$$B = B' \left(\frac{N.A.}{m} \right)^2 \qquad \text{---------- 6 (11)}$$

where B' is the luminance of the souce, m the overall

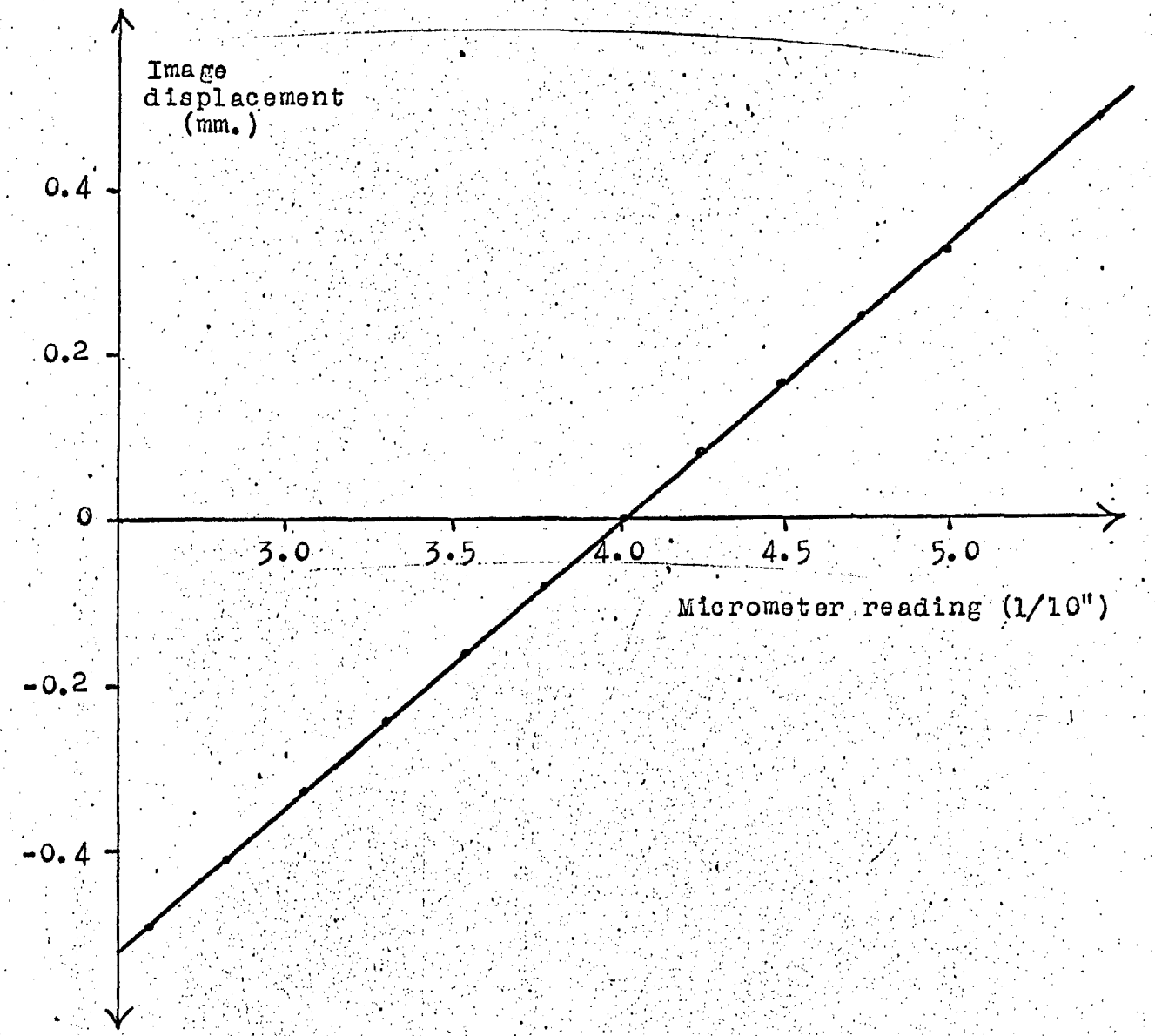


Fig. 6.6. Image movement produced by the scanning block of the direct microscope.

magnification on the screen and N.A, the numerical aperture of the cone of light illuminating the object. Equation 6 (ii) is derived assuming that there are no light losses in the system and that the screen acts as a Lambertian diffuser i.e. its luminance does not vary with the angle of view. In practice however there is light lost in the system due to reflection at each air-glass and glass-air surface, absorption, scattering and dirt on the glass surfaces. Also with a translucent back-projection screen, the luminance is not uniform, but as can be seen from Fig.5.2. is a maximum in the direction of the incident light.

Substituting in equation 6 (ii) the values for the projection microscope, i.e. $B' = 2 \times 10^8$ candelas per square metre, N.A. = 1.3, and m = 1500 X, the theoretical value for the screen luminance is about 160 candelas per square metre, Under the above conditions the screen luminance was measured with an S.E.I. photometer in the normal viewing position, and the value obtained was about 90 candelas per square metre. This showed that the effect of light losses in the system in decreasing the screen luminance, was about twice that of the directional scattering effect of the two ground glass screens, in increasing it in the normal viewing position.

This value of 90 candelas per square metre is high enough to allow the microscope to be used with ambient illumination corresponding to ordinary room lighting, with

a visor across the top of the screen as shown in Fig.6.4, thus permitting quite comfortable viewing. However this level of luminance is achieved by not using a wavelength filter with the mercury source so that the screen images in the white field suffer from transverse chromatic aberration produced by the objective. This can be overcome by using a variable compensating eyepiece, such as one of the Watson Holoscopic series, so that the transverse chromatic aberration of the objective can be exactly matched by suitable adjustment of the lenses in the eyepiece.

6.6. The Sizing Systems of the Projection Microscope

Two different sizing systems were used with the projection microscope. The first was a glass scanning block similar to the one used in the direct microscope, used in conjunction with a single vertical line ruled directly on to the stationary screen. The second was a calibrated wedge also ruled directly on to the screen.

The scanning block unit can be seen in Fig. 6.4. attached to the microscope system, but its action is more clearly illustrated in Fig.6.7. where it is shown in isolation. The glass block was cut from Pilkington plate glass of refractive index 1.52, and mounted on the top of a silver steel rod. The cylindrical hole in the glass was made with an ultra-sonic drill, using a small piece of silver steel cut from the same rod as the drilling tool. The rod is mounted in two bearings, which are fixed to

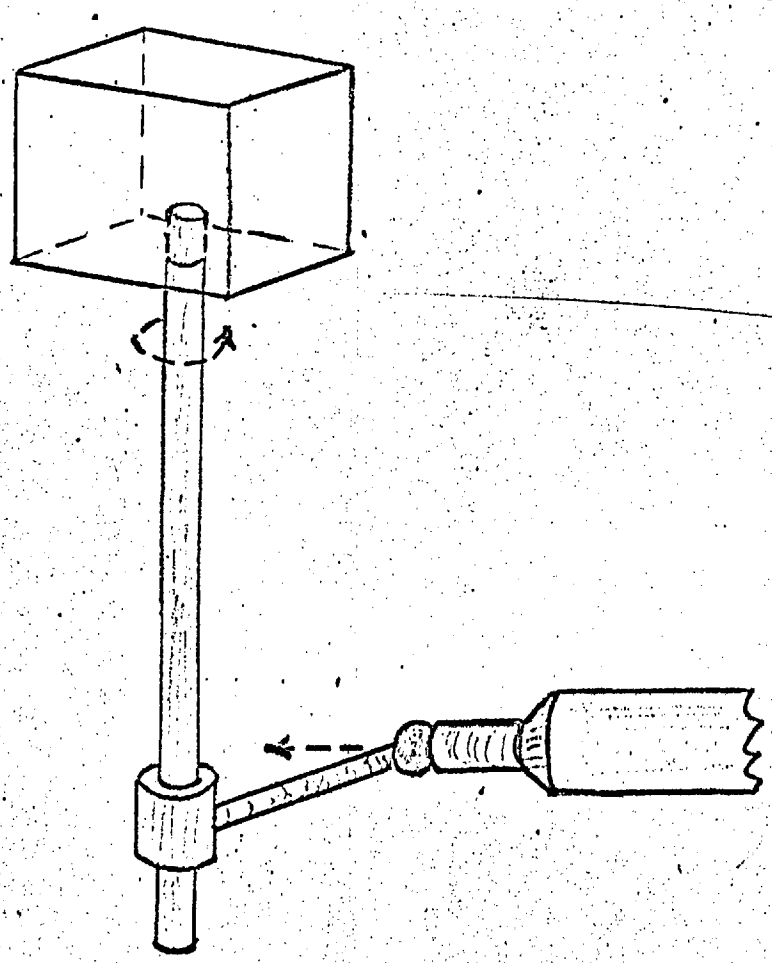


Fig.6.7. The scanning block of the projection microscope

two brass plates, these being clamped to the microscope tube. Near the bottom of the rod is a collar to which is fixed a lever with a metal sphere attached to its end. The sphere makes contact with the free end of a micrometer head, mounted on the projection screen stand, contact being held by a weight on the end of a piece of string attached to the lever, the string passing over a pulley. Movement of the micrometer causes the block to rotate about a vertical axis, so that as the block intercepts the light beam between the eyepiece and the screen, horizontal movement of the image on the screen occurs. The fact that the micrometer head is mounted independently from the microscope stand, ensures once again that there will be no unwanted movement of the image during the sizing process.

The single vertical line is ruled on to the ground surface of the stationary screen with black marking ink, the sizing process being exactly the same as for the direct microscope.

The scanning block is subject to the same conditions as described for the one in the direct microscope, so it will not be necessary to repeat them here. Once again it was found that the optical thickness of the block was constant to within $\frac{\lambda}{4}$ using the Twyman-Green interferometer, and once again it then became necessary to test the linearity of the image movement. The maximum image movement required is 3 mm corresponding to 2 μ in the object space, with a total magnification of about X 1500, this being achieved by a

displacement of 1½ mm to either side of the field.

The linearity of the movement was examined using a X 20 objective, a X 10 eyepiece and a stage micrometer with a spacing of 2 μ. Thus for each movement corresponding to 2μ in the object space the image was displaced 400 μ and for each of these displacements the micrometer reading was noted in tenths of an inch over the whole range. The results are plotted in Fig.6.8. from which it can be seen that over the maximum necessary range of 1½ mm to either side of the centre, there is no detectable systematic deviation from linearity, the average random deviation again being less than 1%. In fact there is no systematic deviation from linearity greater than this 1% random value until about 2½ mm either side of the centre, at which point the second term in equation 6 (i) begins to influence the image movement. Once again over the required range the linearity of movement of the block was found to be quite adequate.

The calibrated wedge is shown in Fig. 6.9. with the scale and the angle greatly exaggerated to illustrate its action. It consists of a pair of converging lines drawn with black marking ink on the stationary ground glass screen with the ratio of the lengths PQ and PR to the length QR approximately 10:1. If the image of an opaque disc is run in between the lines until it just appears to touch both as in Fig. 6.9. then its size can be read off the scale.

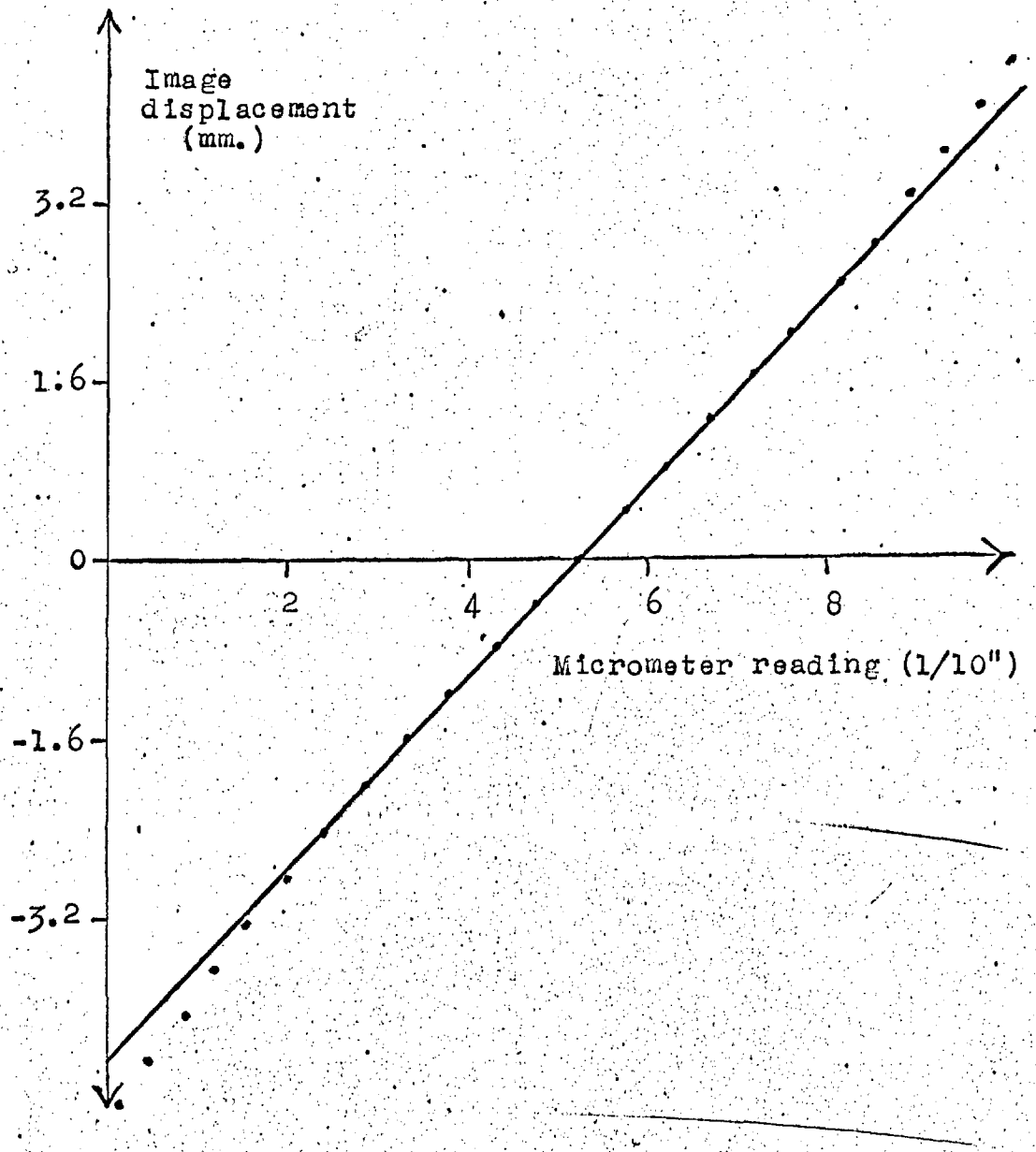


Fig. 6.8. Image movement produced by the scanning block of the projection microscope.

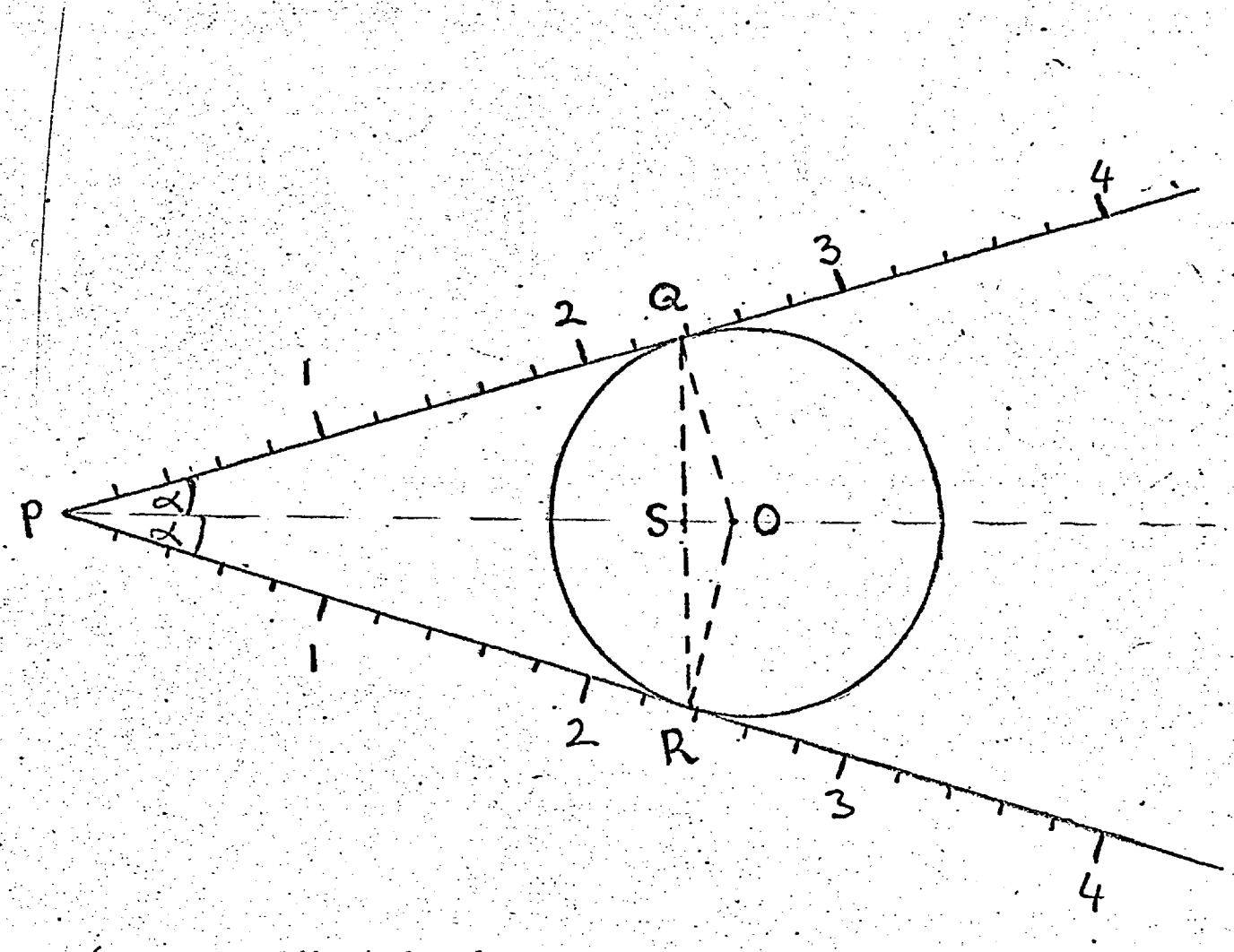


Fig. 6.9. The calibrated wedge

With the ratio at about 10:1 it then becomes possible to estimate the reading to a further significant figure than would be the case if the same linear scale was used normally.

To calibrate the wedge it is first necessary to measure the lengths PQ, PR and QR accurately, with a travelling microscope, so that the exact value of the ratio is known, and then to compare the scale with a stage micrometer. This would then give the actual size of the length Q R in terms of the scale, and not the diameter of the disc. However from the diagram it can be seen that the ratio of the actual diameter to the measured length is QO/QS and that

$$QO = PQ \tan\alpha \quad \text{and} \quad QS = PQ \sin\alpha$$

$$\text{thus} \quad QO/QS = \text{Sec}\,\alpha$$

$$\text{Now } \sin\alpha = 0.05 \quad \text{thus } \alpha = 3^{\circ}$$

$$\text{Thus } QO/QS = 1.001$$

So that using this measurement gives a result which has a systematic error of about -1/10%, which is certainly small enough to justify the fact that no correction factor need by applied.

CHAPTER 7

THE VISUAL SIZE OF THE OPAQUE DISCS WITH THE DIRECT MICROSCOPE

7.1 Introduction

In section 2.4. an account was given of the effect of the optical and visual factors on the visual sizing of opaque discs by direct microscopy, as determined by Charman (1961,1963(a) and 1963(b)). As Charman's investigation was completed a repeat of the same will not be the aim of this chapter. Contained here will simply be the description of sufficient experiments on the visual sizing of the discs with the scanning block of the direct microscope, so that the results from these experiments may be compared with :-

(a) results from experiments with the filar micrometer eyepiece (also to be described in this chapter).

(b) results from experiments with the scanning block of the projection microscope (to be described in the following chapter).

7.2. Results Using the Scanning Block

The primary image of an opaque disc has a continous luminance distribution almost identical to those in Fig.4.4., but the eye sees the disc image as effectively a dark disc in a bright surround, whose subjective brightness may vary across its diameter but whose edges are fairly sharply defined. In measuring the visual diameter the

edges used are those of the dark disc, where the image brightness is just noticeably lower than that of its bright surround. The image is first set tangentially to one side of the vertical line graticule, and is then traversed across the line, until the small dark area which is moving towards it just vanishes. The same edge of the vertical line is then used in both cases, eliminating the effect of its finite width. The difference between the scanning block micrometer readings in these two positions, multiplied by a calibration factor gives the 'visual size' of the image.

The conditions under which the visual sizing with the scanning block was performed were as follows:-
The wavelength of the illuminating light $\lambda = 0.546\mu$, the N.A. of the objective was 1.33, and that of the condenser 1.27. The primary magnification of the objective at its optimum tubelength was about 90X, which with a 20X eyepiece gives a total magnification of the order of 1800X. The retinal illumination in the clear areas of the field was varied slightly, and had values of 10^2, 10^3, 10^4, trolands, these being determined by setting a white diffusing screen at a known distance from the eyepiece, and measuring its luminance with an S.E.I. photometer.

The visual sizing was carried out for different disc diameters, 65 readings being taken in each case. The results are shown in Table 7 (i), $\bar{X}$ being the mean disc diameter, $\bar{Z}$ the mean visual size and S'' its standard

Table 7 (i) Visual sizing of opaque discs with the scanning block of the direct microscope

Rot. Ill.	10^2 Trolands		10^3 Trolands		10^4 Trolands	
$\bar{X}$	$\bar{Z}$	S''	$\bar{Z}$	S''	$\bar{Z}$	S''
0.264	0.413	0.078	0.482	0.064	0.518	0.062
0.365	0.500	0.050	0.553	0.072	0.568	0.086
0.511	0.548	0.060	0.598	0.056	0.646	0.090
0.814	0.863	0.052	0.898	0.068	0.913	0.080
1.171	1.168	0.088	1.202	0.098	1.241	0.125

deviation. Figs. 7.1, 7.2., and 7.3., show the visual size plotted against the true size of the discs for retinal illuminations of 10^2, 10^3, and 10^4, trolands respectively, all the other conditions being kept constant as described above. The broken line is drawn at 45° to either axis and the vertical ranges shown correspond to plus and minus the standard deviation about the mean.

Comparison of these three sets of results as shown in Fig. 7.4. in which smooth curves are drawn through the mean values confirms for the whole size range the trend found by Charman (1963 (a)), namely that the visual size increases with increasing retinal illumination, and which he explained by considering the luminance discrimination of the eye. This trend is also consistent with the results found by Watrasiewicz (1966) in investigating the Mach effect in the image of an opaque straight edge. He also showed that with increasing retinal illumination, the visual edge moves away from the geometrical edge of the image.

All three curves in Fig.7.4. indicate that the visual size of the opaque discs is no longer usefully related to the true size where the object diameter falls bellow about 0.4μ, which fact was found by Charman (1963(b)) for the visual sizes of circular holes in an opaque surround. The visual size of the discs having diameters below this level tends to a constant value dependent on the level of retinal illumination. On the other hand when the disc diameter is

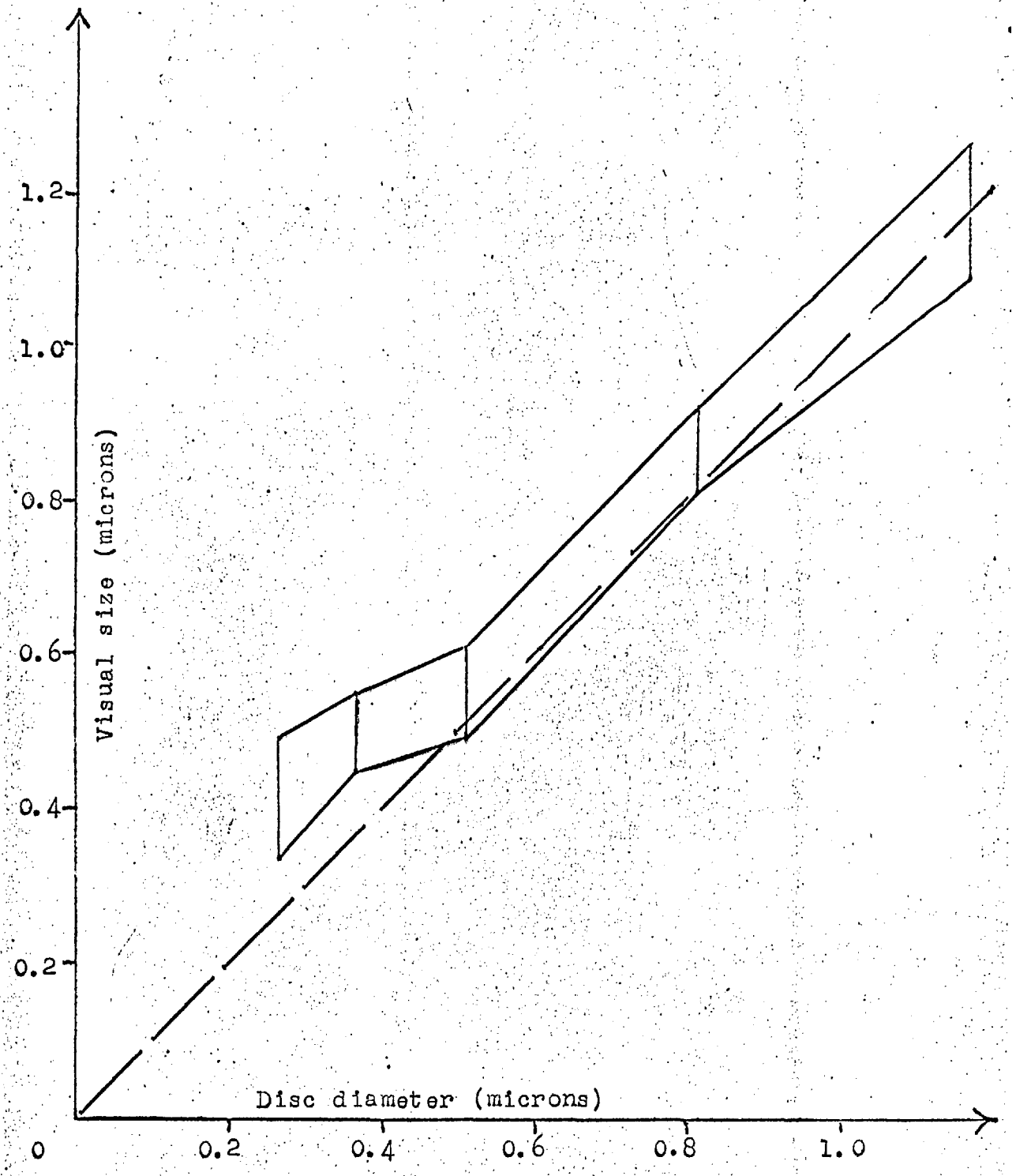


Fig.7.1. Variation of visual size with true diameter for opaque discs sized with the scanning block of the direct microscope. $\lambda = 0.546\mu$, objective N.A. = 1.33, condenser N.A. = 1.27, magnification = 1800X, retinal illumination in clear areas of field = 10^2 trolands.

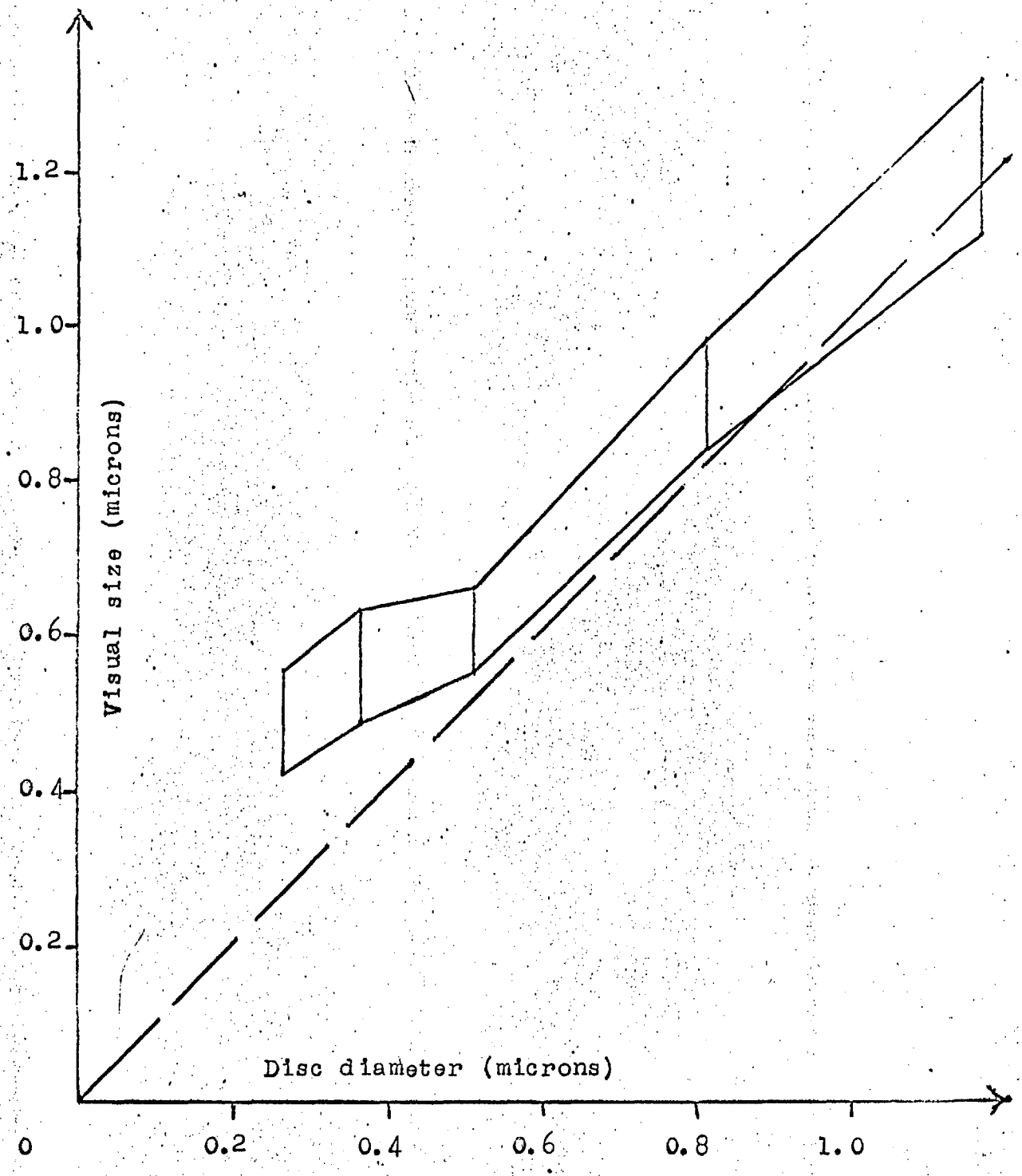


Fig.7.2. Variation of visual size with true diameter for opaque discs sized with the scanning block of the direct microscope. $\lambda = 0.546\mu$, objective N.A.=1.33, condenser N.A. = 1.27, magnification = 1800X, retinal illumination in clear areas of field = 10^2 trolands.

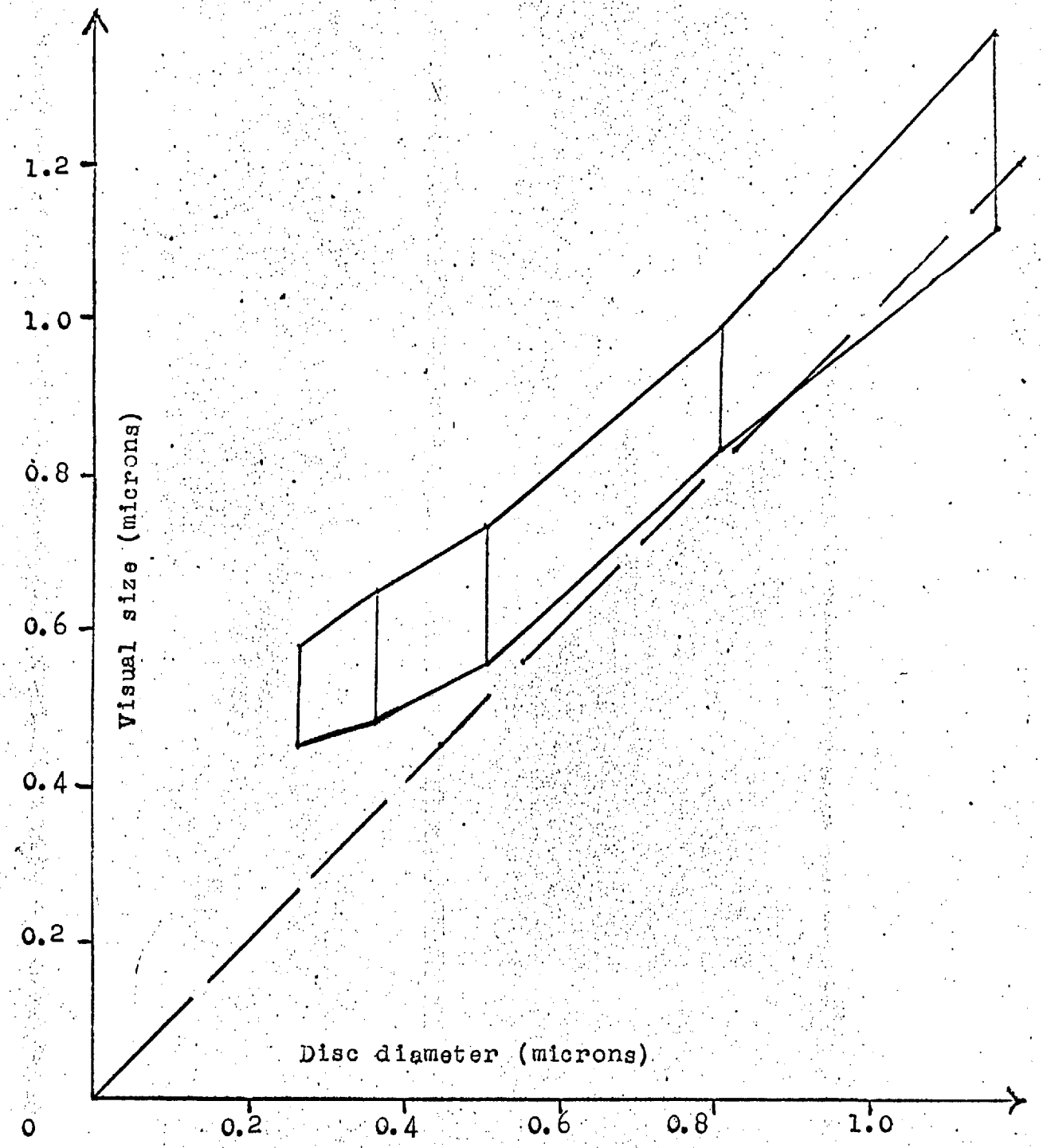


Fig.7.3. Variation of visual size with true diameter for opaque discs sized with the scanning block of the direct microscope. $\lambda = 0.546\,\mu$, objective N.A. = 1.33, condenser N.A. = 1.27, magnification = 1800X, retinal illumination in clear areas of field = 10^4 trolands.

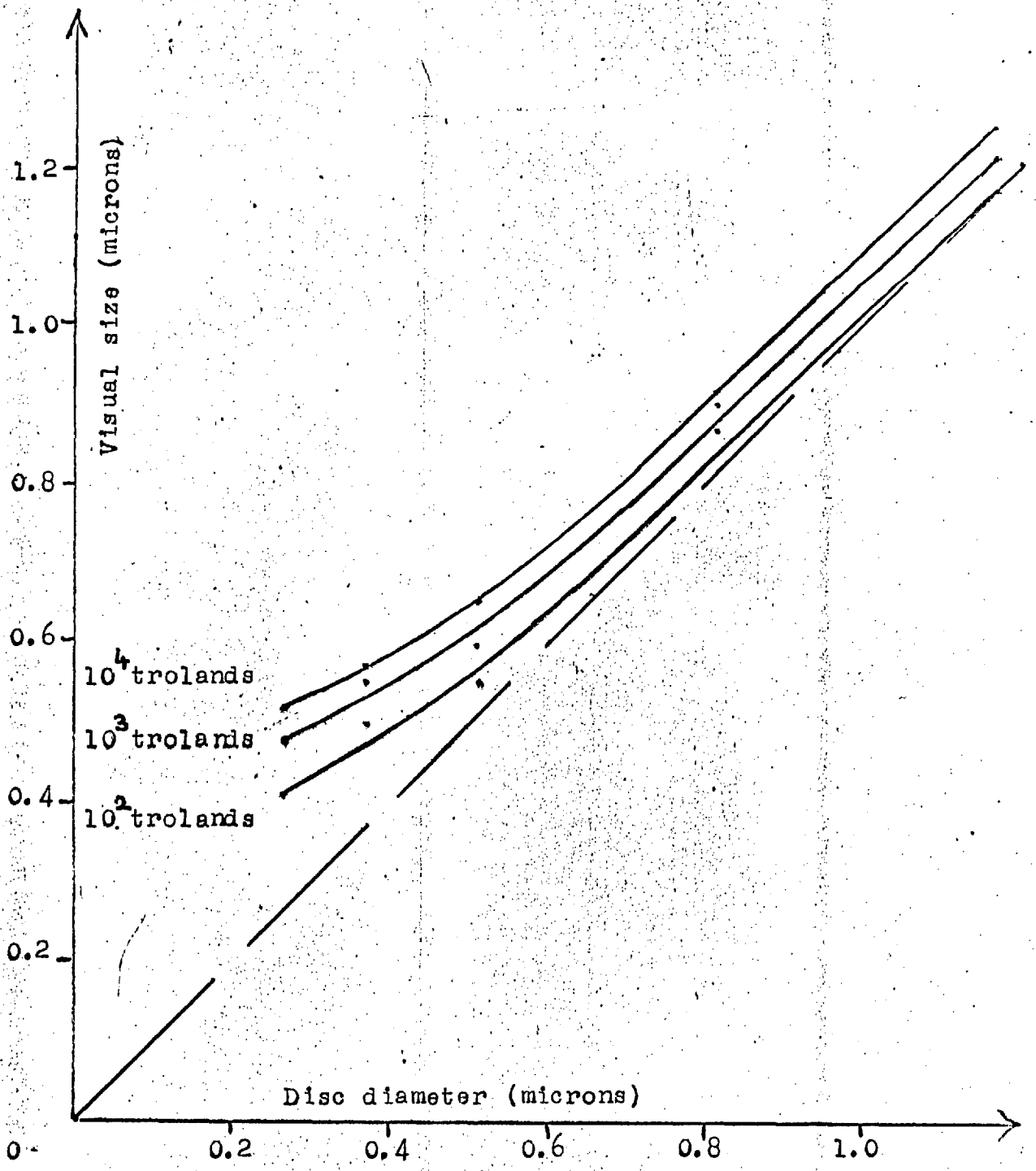


Fig.7.4. Variation of visual size with true diameter for opaque discs sized with the scanning block of the direct microscope. $\lambda = 0.546\,\mu$, objective N.A. = 1.33, condenser N.A. = 1.27, magnification = 1800X, retinal illuminations in clear areas of field as indicated.

larger than 0.4μ the visual size is fairly consistently in excess of the true size by an amount which is also dependent on the retinal illumination level.

7.3. Results Using a Filar Micrometer Eyepiece.

Using the visual part of the photoelectric micrometer microscope described in Chapter 4, the opaque discs were sized using a filar micrometer eyepiece, under exactly the same optical conditions as their diffraction images were determined using the photoelectric part. Rotation of a graduated micrometer drum on the eyepiece results in the movement of a single crosswire with respect to a fixed graticule, therefore the sizing technique used is exactly the same as with the scanning block, except that in this case the disc remains stationary and the crosswire is moved. Again the edges used are those of the dark disc, where the image brightness is just noticeably lower than that of its bright surround. To size, the eyepiece crosswire is first set tangentially to one side of the image and is then traversed across the image disc until the small dark area towards which the crosswire is moving just vanishes, the same edge of the crosswire being used in both cases.

The conditions under which the above visual sizing was performed were as follows:-

The wavelength of the illuminating light $\lambda = 0.53 \pm 0.01\mu$, and the N.A.'s of the objective and condenser were both equal to 1.28. The primary magnification of the objective at its optimum tubelength was 96X, which with a 20X eyepiece

gave a total magnification of about 1900X. The retinal illumination in the clear areas of the field was measured to be 3×10^4 trolands.

As before 65 determinations were made for each different disc diameter, the results being summarized in Table 7 (ii). $\bar{X}$, $\bar{Z}$ and S''' are as before and $\bar{Tv}$ is the mean transmittance at the visual edge of the image, given together with its approximate variation. The positions of the visual edges on the transmission curves for the images are shown in Fig.7.5., which is simply Fig.4.4. with the addition of a further set of short vertical lines above the other two, representing these visual edges. It can be seen that as the size of the disc increases, thus causing a slight decrease in the effective luminance over the central area of the field, the luminance interval between the clear part of the field and the visual edge increases. This trend agrees qualitatively with the luminance discrimination data for the eye given in Fig.2.3. but no quantitative deductions are possible, because of the difficulty in estimating the above mentioned effective luminance.

Figs. 7.6. shows the visual size plotted against the true size of the discs, and Fig.7.7. shows these results compared with a set of those obtained using the scanning block of the direct microscope. The set chosen were those which were obtained under conditions as near as possible to the conditions described in this section, namely with a retinal illumination of 10^4 trolands, the rest of the conditions being

Table 7 (ii) Visual sizing of opaque discs with a filar micrometer eyepiece

$\bar{x}$ μ	$\bar{z}$ μ	s''' μ	$\bar{T}v$ %
0.264	0.539	0.067	89 ± 5
0.365	0.595	0.057	83 ± 6
0.511	0.693	0.063	82 ± 5
0.814	0.938	0.053	64 ± 6
1.171	1.272	0.075	63 ± 5

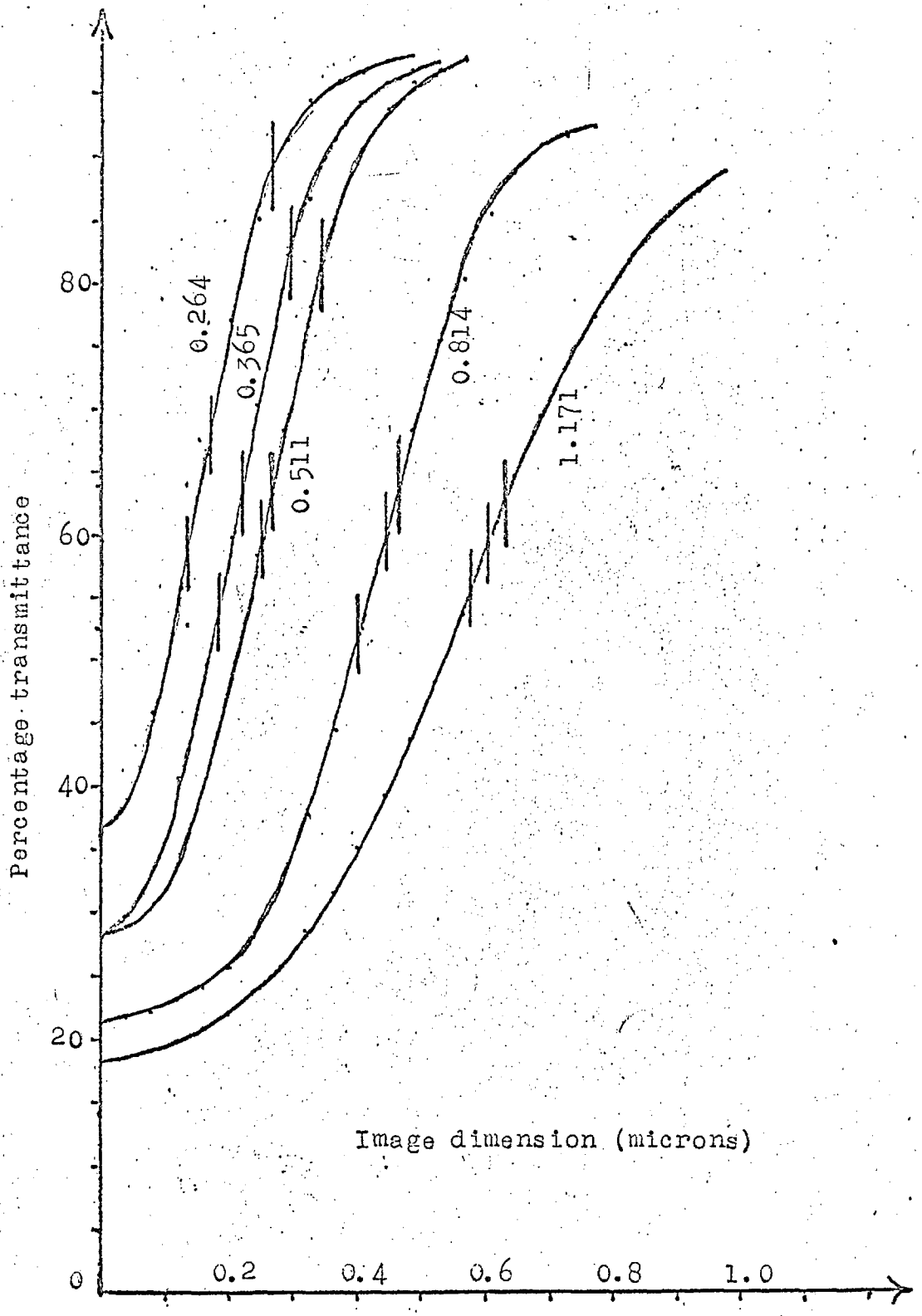


Fig.7.5. The images in full-cone illumination (S=1) of opaque discs having the diameters indicated. Condenser and objective N.A.'s = 1.28, $\lambda = 0.53\mu$, $\delta\lambda = 0.01\mu$. The upper, middle and lower sets of short vertical lines on the curves correspond to the positions of the visual edges, the image half-widths and the geometrical edges respectively.

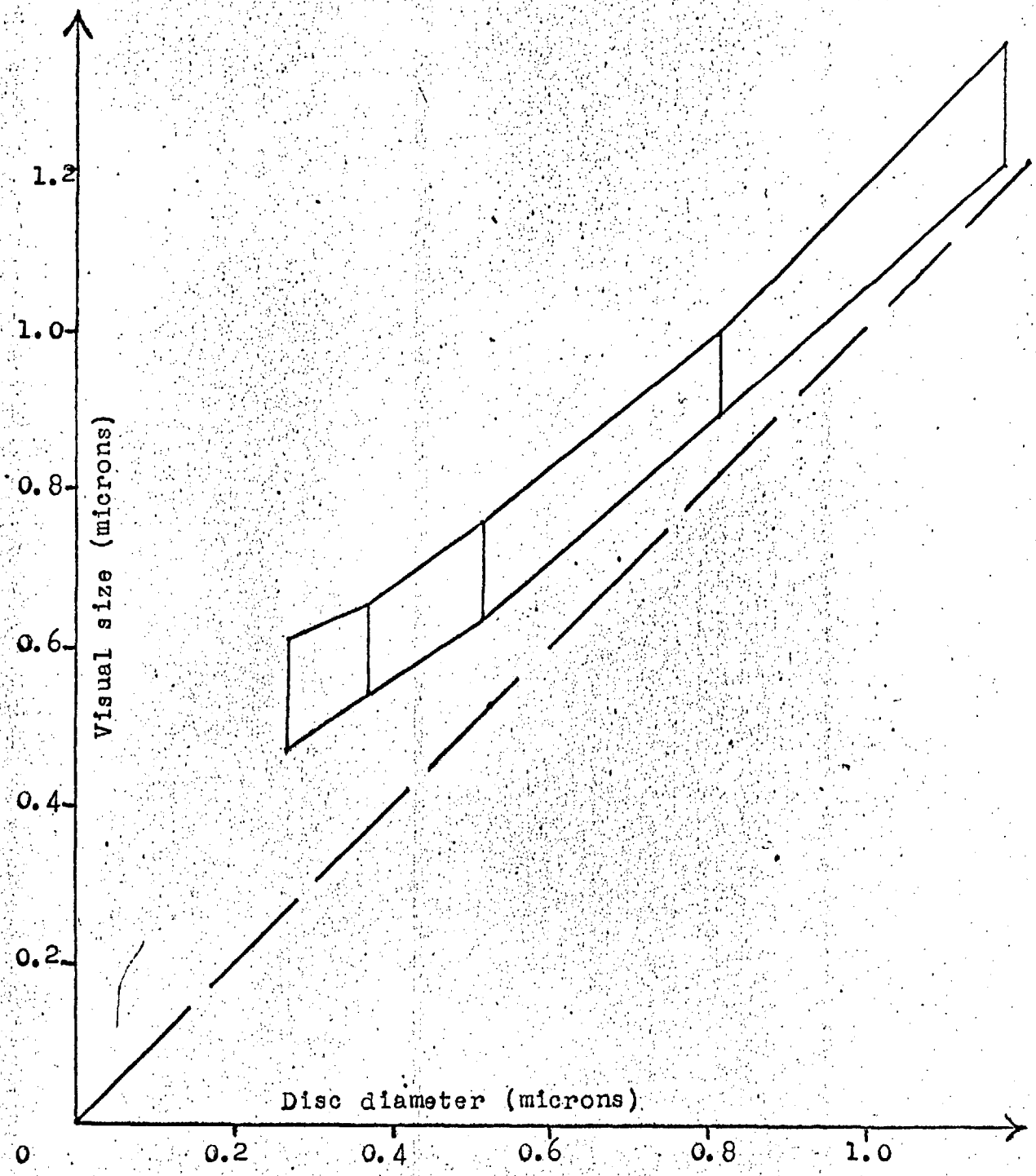


Fig.7.6. Variation of visual size with true diameter for opaque discs sized with a filar micrometer eyepiece. $\lambda = 0.53 \pm 0.01 \mu$, objective and condenser N.A.'s = 1.28, magnification = 1900X, retinal illumination in clear areas of field = 3×10^4 trolands.

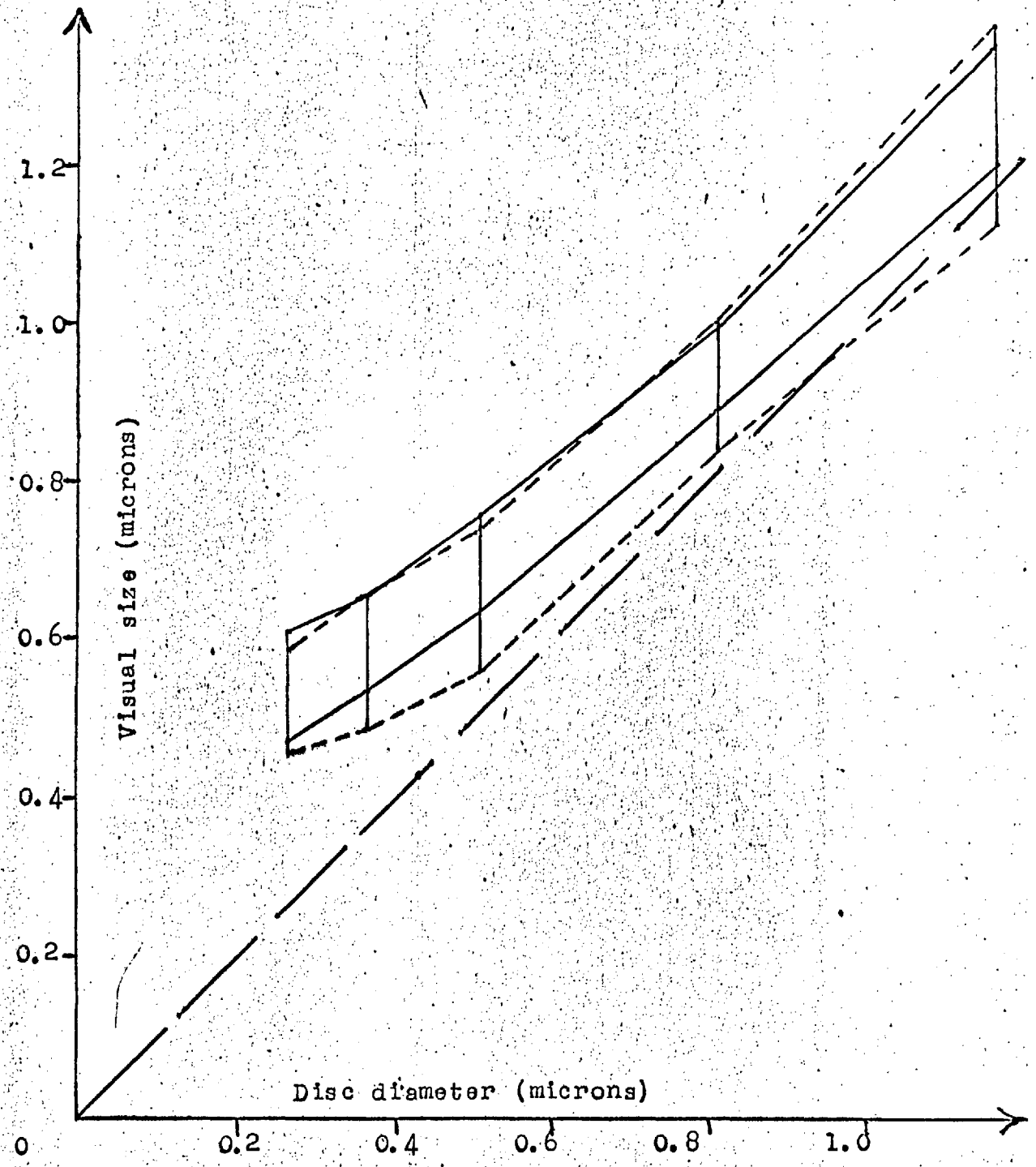


Fig.7.7. Variation of visual size with true diameter for opaque discs sized with (a) a filar micrometer eyepiece (results from Fig.7.6. plotted with continuous line), (b) the scanning block of the direct microscope (results from Fig.7.3. plotted with broken line).

as described in the previous section, these results having previously been plotted in Fig.7.3. In Fig.7.7., the results from Fig.7.6. are plotted with the continous line, and those from Fig.7.3. with the broken line. A comparison of the two shows that those obtained with the filar eyepiece are slightly larger over the whole range. This is easily explained by the fact that these results were obtained with a retinal illumination greater by a factor of 3, the increase in size simply being a continuation of the trend illustrated in Fig.7.4. Comparison also shows that the standard - deviations of the results with the filar eyepiece are consistently less than those with the scanning block showing that the former has a slightly better setting accuracy. Apart from the difference by a factor of 3 in the retinal illumination, the conditions under which the two sets of results plotted in Fig.7.7. were obtained were marginally different. However it should be noted that although the N.A.'s given were very similar, two different microscope objectives were used, both being well corrected. Charman (1963 (d)) has shown that different objectives having similar quality and aperture produce, within the limits of experimental error, identical images this fact being confirmed by the work of Watrasiewicz (1965(b)). Hence the similarity between the two sets of results plotted in Fig.7.7. obtained with the two different objectives.

As with the curves in Fig.7.4., the results in Fig.7.7

show that the visual size of the discsis no longer usefully related to the true size where the object diameter falls below about 0.4. μ (the upper curve in Fig.7.4. and the broken lines in Fig.7.7. representing of course the same set of results). The visual size of discs having diameters below this level again tends to a constant value dependent on the level of retinal illumination. Also when the disc diameter is larger than 0.4. μ the visual size is fairly consistently in excess of the true size by an amount which is also dependent on the retinal illumination level. For a comfortably bright image field, as is the case with the results in Fig.7.7. this amount is about 0.12 μ (i.e. approx 0.5. Airy units) and is accurate to about $\pm$ 0.063 μ in the case of the filar micrometer eyepiece and $\pm$ 0.076 μ in the case of the scanning block and line graticule (i.e. approx. $\pm$ 0.25 and $\pm$ 0.30 Airy units respectively).

CHAPTER 8

THE VISUAL SIZE OF THE OPAQUE DISCS WITH THE PROJECTION MICROSCOPE

8.1. Introduction

The opaque discs were sized on the projection screen using the two systems described in Section 6.6. With the first system the scanning block, a series of experiments were carried out on the effects of different operating conditions, variations being made in the spectral composition of the light, the luminance of the projection screen, and the setting of the microscope condenser. With the second system the calibrated wedge, experiments were first carried out to investigate its use as a direct reading instrument and then to compare its sizing results with those of the scanning block.

8.2. Results Using the Scanning Block

In measuring the visual diameter of the opaque discs on the projection screen using the scanning block exactly the same technique is used as was used with the scanning block of the direct microscope. The only difference is that in this case as the vertical line is ruled directly on to the screen it will not suffer the slight diffraction effects of the vertical line graticule viewedthrough the eyepiece, as was the case previously.

The conditions under which the first set of visual

sizing results were obtained by this method were as follows:- The mercury source was used with out the wavelength filter so that the field appeared white and the range of wavelengths were those of the whole visible mercury spectrum, the extreme violet and yellow lines having wavelengths of 0.405 μ and 0.579 μ respectively. The N.A. of the objective was 1.33 and that of the condenser 1.27. The primary magnification of the objective at its optimum tubelength was about 90X, which with a 20X holoscopic eyepiece, adjusted for zero lateral chromatic aberration, gave a total magnification on the screen of the order of 1500X. The screen luminance was set at 9 candelas per square metre, by placing a 10% transmitting neutral filter in front of the source this being checked with the S.E.I. photometer.

65 determinations were made for each different disc diameter, the results being summarized in Table 8 (i) and plotted in Fig.8.1.

In order to change the spectral composition of the light, and keep the screen luminance roughly at the same level, the neutral filter was removed and the green one was put in its place. This now meant that the wavelength of the light $\lambda = 0.546 \mu$, and the screen luminance as measured with the S.E.I. photometer was found to be 18 candelas per square metre. The rest of the operating conditions were kept constant. Again 65 determinations were made for each different disc diameter, and the results are summarized in Table 8 (ii).

Table 8 (i) **Visual sizing of opaque discs with the scanning block of the projection microscope.**

Mercury (white) light (λ min = 0.405μ, λ max = 0.579μ)

Condenser N.A. = 1.27 Screen luminance = 9cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	s'' μ
0.264	0.419	0.069
0.365	0.463	0.062
0.511	0.527	0.049
0.814	0.845	0.082
1.171	1.177	0.103

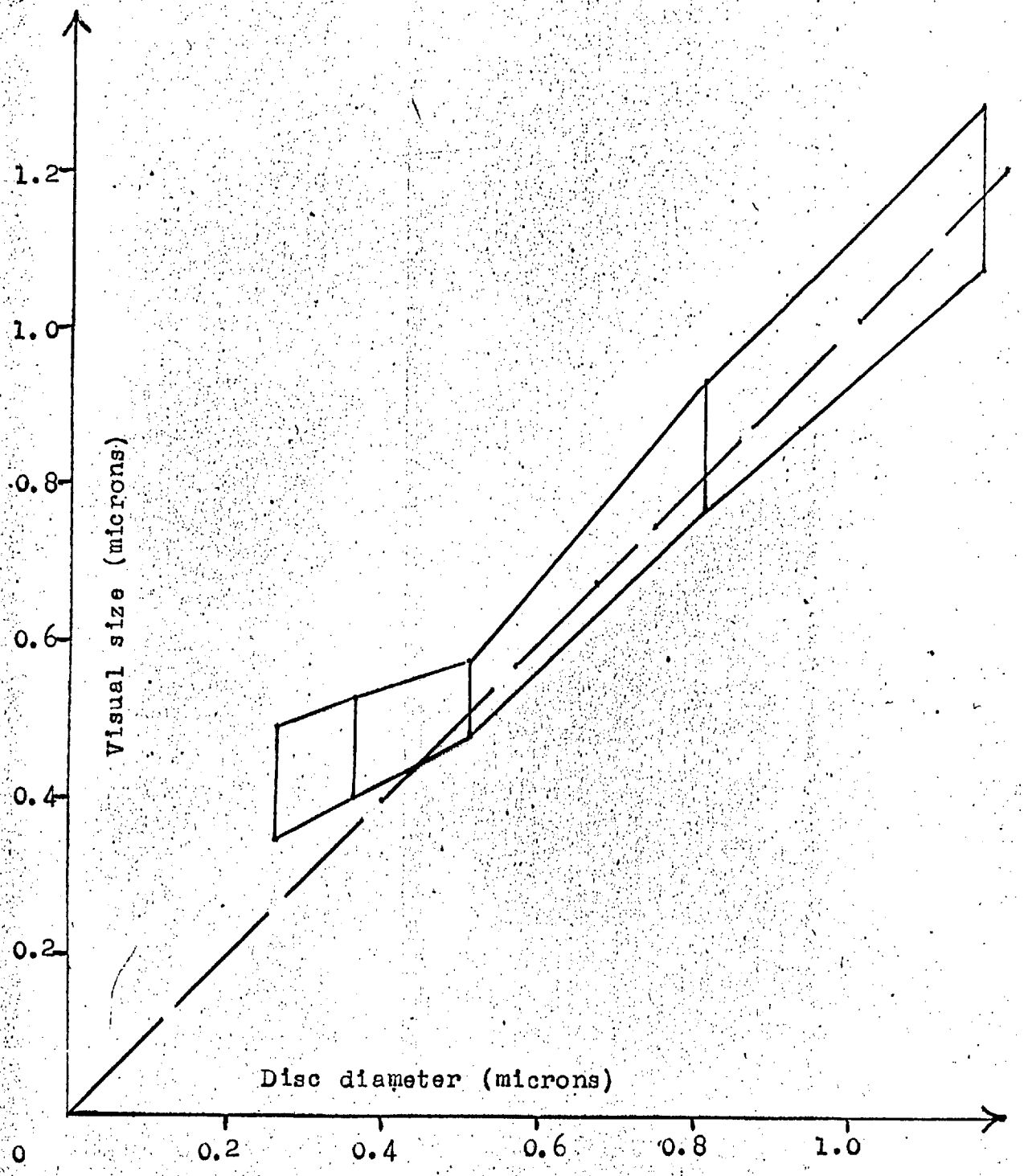


Fig.8.1. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. Mercury white light (λmin. = 0.405μ, λ max = 0.579μ), objective N.A. = 1.33, condenser N.A. = 1.27, magnification = 1500X, screen luminance = 9 cd/m^2.

Table 8 (ii) Visual sizing of opaque discs with the scanning block of the projection microscope

Green light ($\lambda = 0.546\ \mu$)

Condenser N.A. = 1.27

Screen luminance = $18 cd/m^2$

$\bar{X}$ μ	$\bar{Z}$ μ	S''' μ
0.264	0.396	0.095
0.365	0.464	0.068
0.511	0.541	0.055
0.814	0.873	0.072
1.171	1.235	0.115

Fig 8.2. shows these results plotted, and Fig.8.3. shows them compared with the previous set plotted in Fig.8.1. In Fig. 8.3. the results from Fig.8.2 are plotted with the continous line and those from Fig.8.1. with the broken line. A comparison of the two shows that over most of the size range the sizes obtained with the green filter were slightly larger. This can easily be explained by the fact that in this case the screen luminance was greater by a factor of 2, indicating that the change in the spectral composition of the light that was made had virtually no effect on the visual size. The explanation of this probably lies in the criterion used fro adjusting the holoscopic eyepiece for zero lateral chromatic aberration. This was that there should be no coloured edges on the images of the discs, meaning that the visual edges for each of the different coloured images were made to be coincident. Under these circumstances therefore it would not be possible to detect any variation in the measured visual size with wavelength.

By removing the green filter it was then possible to investigate in isolation, the effect of increased luminance. Mercury (white) light was used, and the luminance of the screen was 90 candelas per square metre, all the other conditions being constant. 65 determinations were made for each different disc diameter and the results are summarized in Table 8 (iii), and plotted in Fig. 8.4. Fig.8.5. shows them compared with those from Fig.8.1. for which the

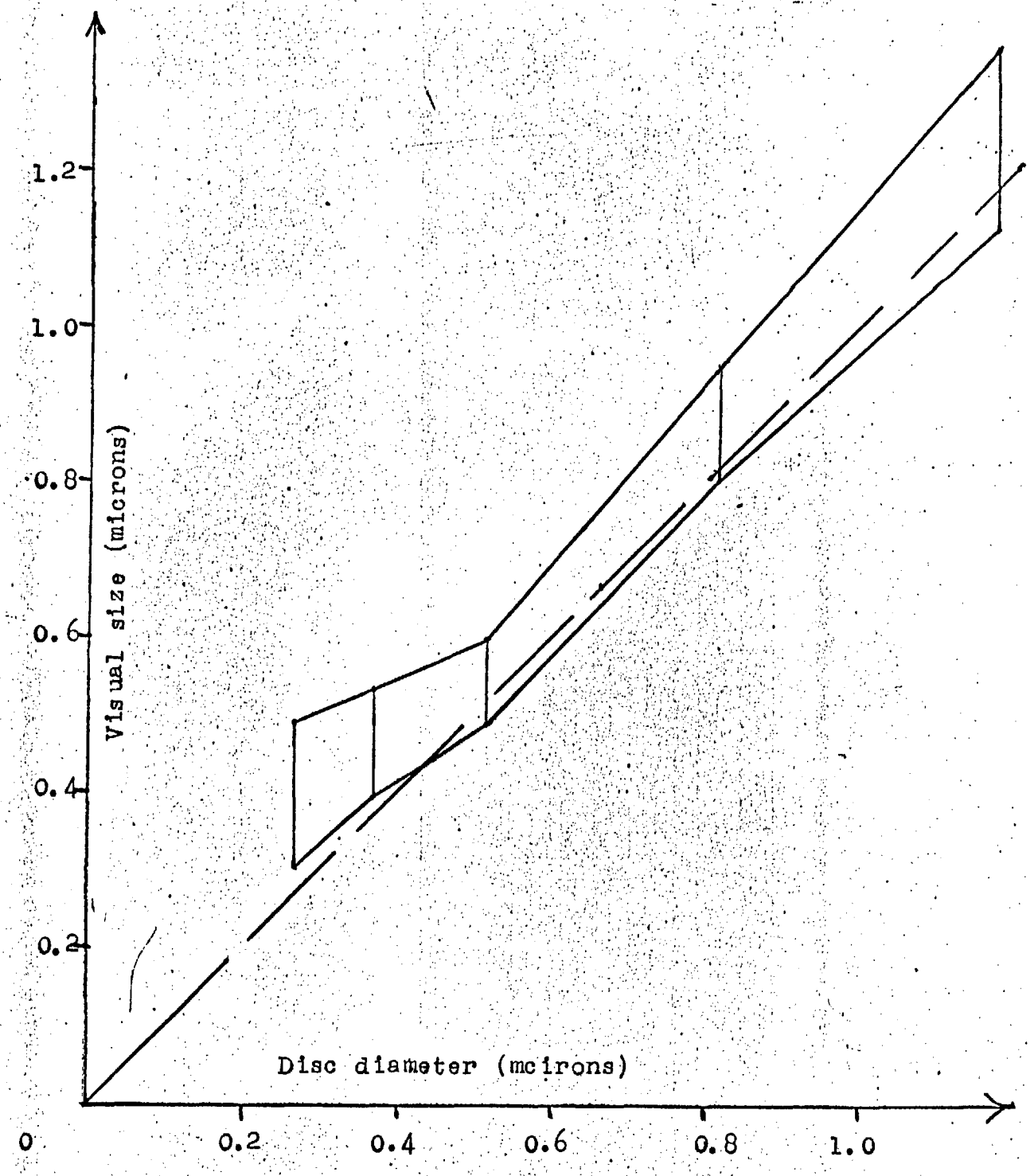


Fig. 8.2. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. Green light ($\lambda = 0.546\mu$), objective N.A. = 1.35, condenser N.A. = 1.27, magnification = 1500X, screen luminance = 18 cd/m^2.

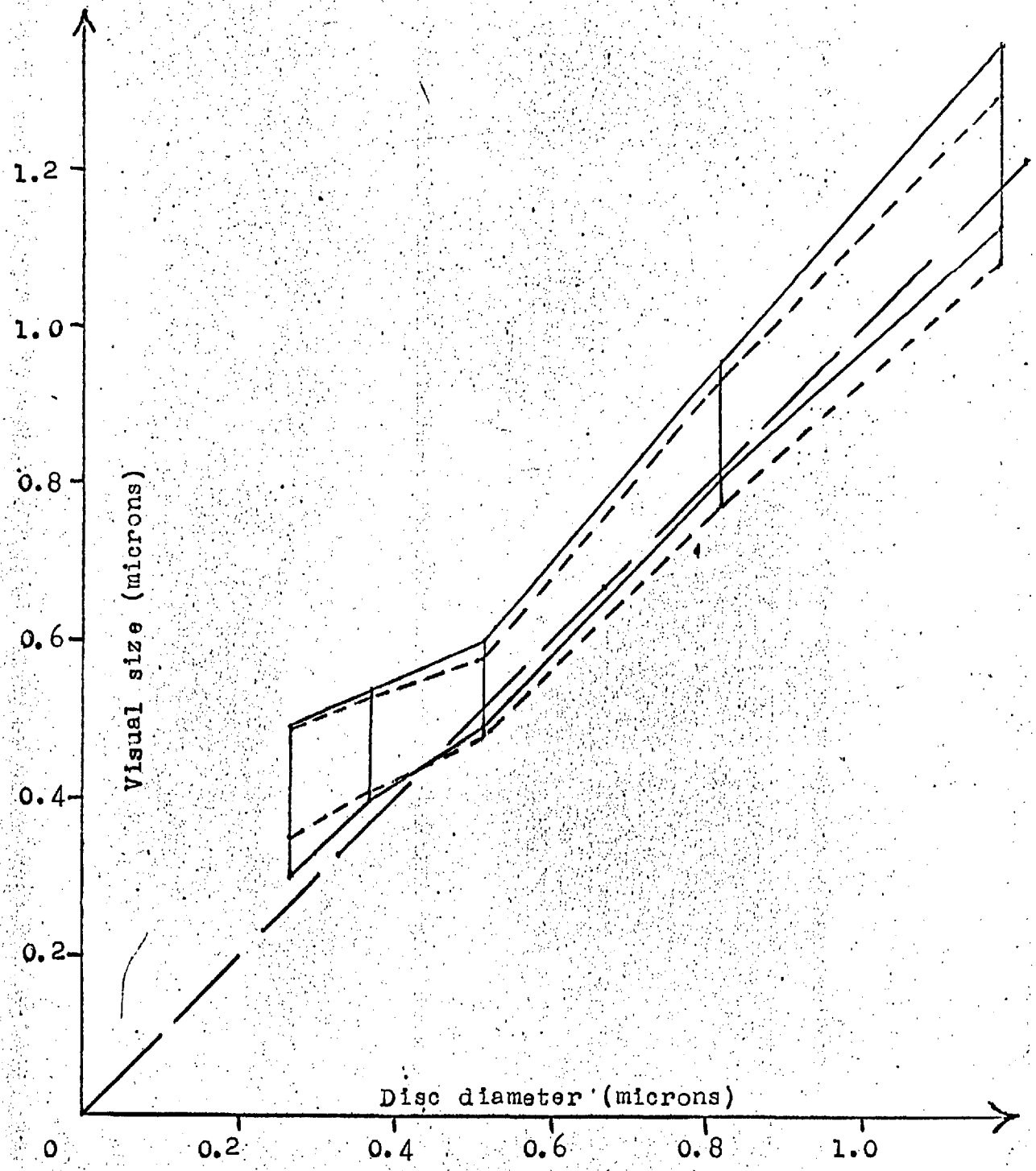


Fig.8.3. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. (a) Mercury green light, screen luminance = 18 cd/m^2 (results from Fig.8.2. plotted with continuous line), (b) mercury white light, screen luminance = 9 cd/m^2, (results from Fig.8.1. plotted with broken line).

Table 8 (iii) Visual sizing of opaque discs with the scanning block of the projection microscope

Mercury (white) light

Condenser N.A. = 1.27

Screen luminance = 90 cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	S_{III} μ
0.264	0.438	0.071
0.365	0.506	0.067
0.511	0.575	0.042
0.814	0.933	0.071
1.171	1.259	0.102

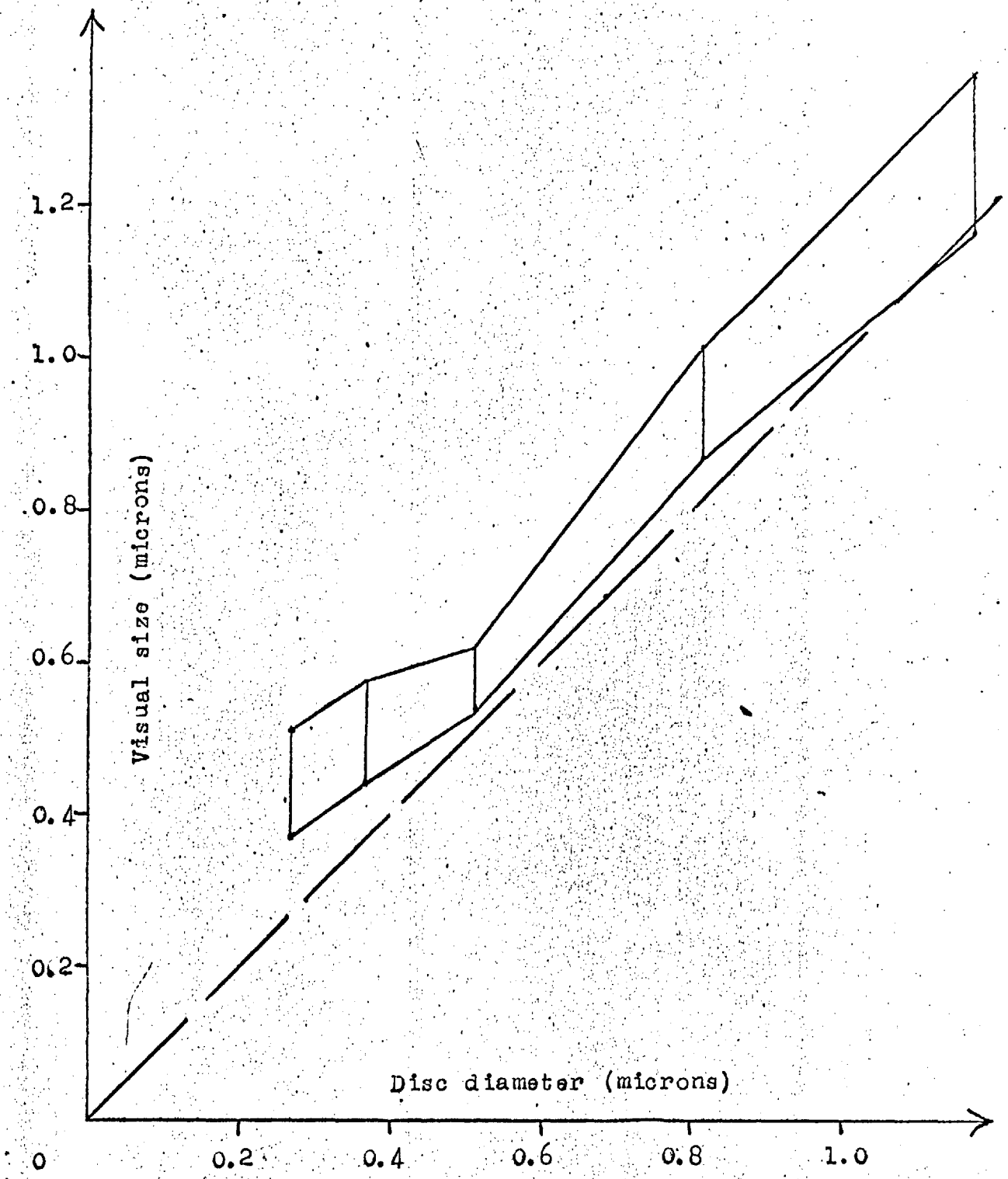


Fig.8.4. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. Mercury white light, objective N.A. = 1.33, condenser N.A. = 1.27, magnification = 1500X, screen luminance = 90 cd/m^2.

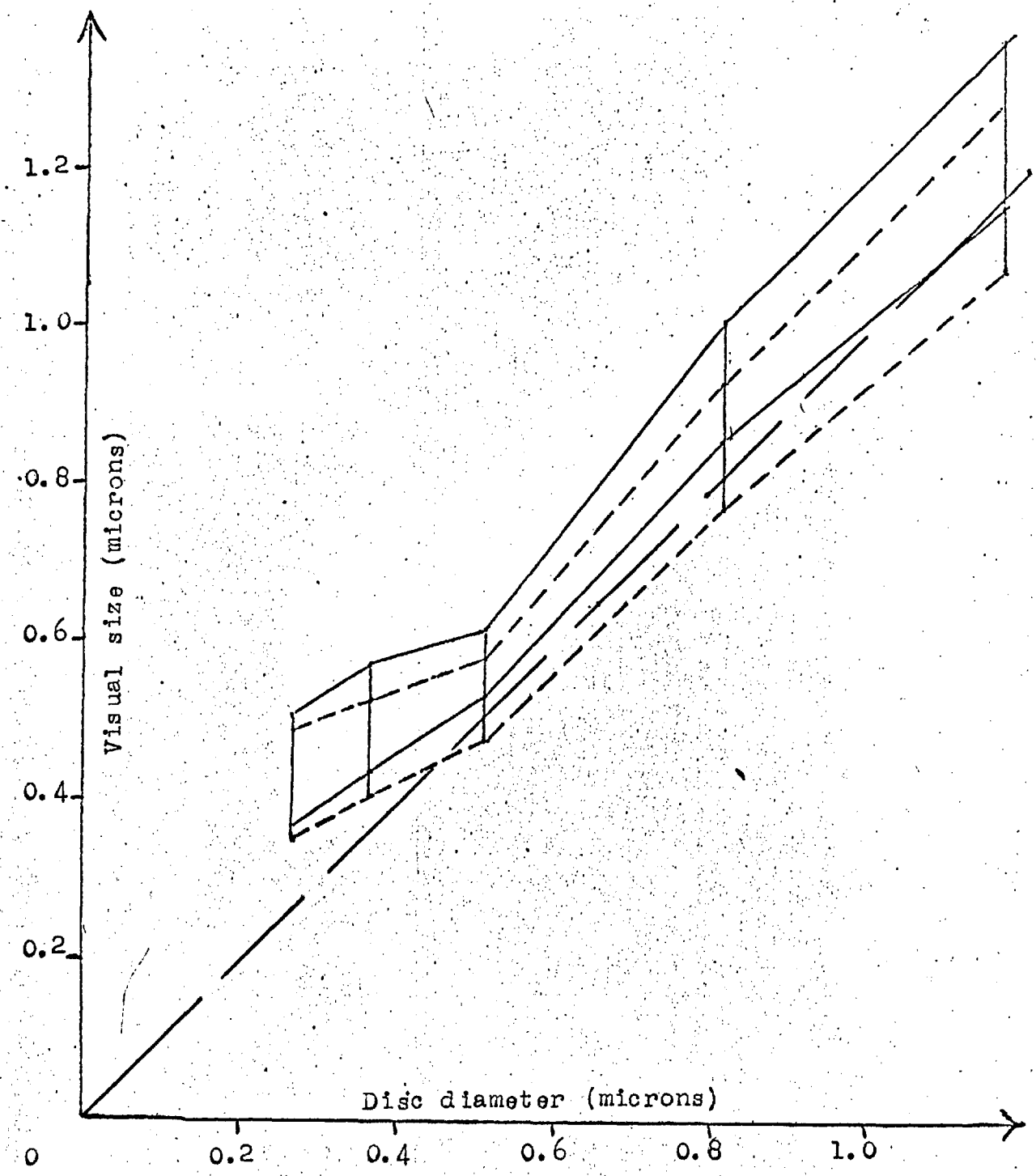


Fig.8.5. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. (a) Screen luminance $\simeq$ 90 cd/m^2(results from Fig.8.4. plotted with continuous line), (b) screen luminance $\simeq$ 9 cd/m^2(results from Fig.8.1. plotted with broken line).

luminance was lower by a factor of 10, the latter being plotted with broken lines. Comparison shows as expected an increase in size with screen luminance over the whole size range.

In order to investigate the effect of lowering the condenser aperture, the N.A. of the condenser was set at 0.37, at which value the screen luminance was found to be 9 candelas per square metre. As before 65 determinations were made for each different disc diameter and the results are summarized in Table 8 (iv) and plotted in Fig.8.6. Fig 8.7. shows them compared with those from Fig.8.1. for which the condenser N.A. was 1.27, and the screen luminance was the same 9 candelas per square metre, the latter being plotted with broken lines. Comparison shows that over the whole size range, there is a substantial increase in visual size with the decreased condenser N.A. This is the same result as that found by Charman (1963(b)) for 1.171 μ discs using a direct microscope, and is in agreement with the trend from curve (2) to curve (1) in Fig.4.2., which shows the change in the diffraction image of a small opaque disc to be expected with decreased condenser aperture.

This phenomenon of increasing visual size with decreasing condenser N.A. for an opaque disc is also consistent with the results found by Watrasiewicz (1963) and Charman and Watrasiewicz (1964) in investigating the Mach effect in the image of an opaque straight edge. They found that the

Table 8 (iv) Visual sizing of opaque discs with the scanning block of the projection microscope.

Mercury (white) light

Condenser N.A. = 0.37

Screen luminance = 9 cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	s''' μ
0.264	0.457	0.068
0.365	0.524	0.068
0.511	0.606	0.056
0.814	0.986	0.077
1.171	1.292	0.120

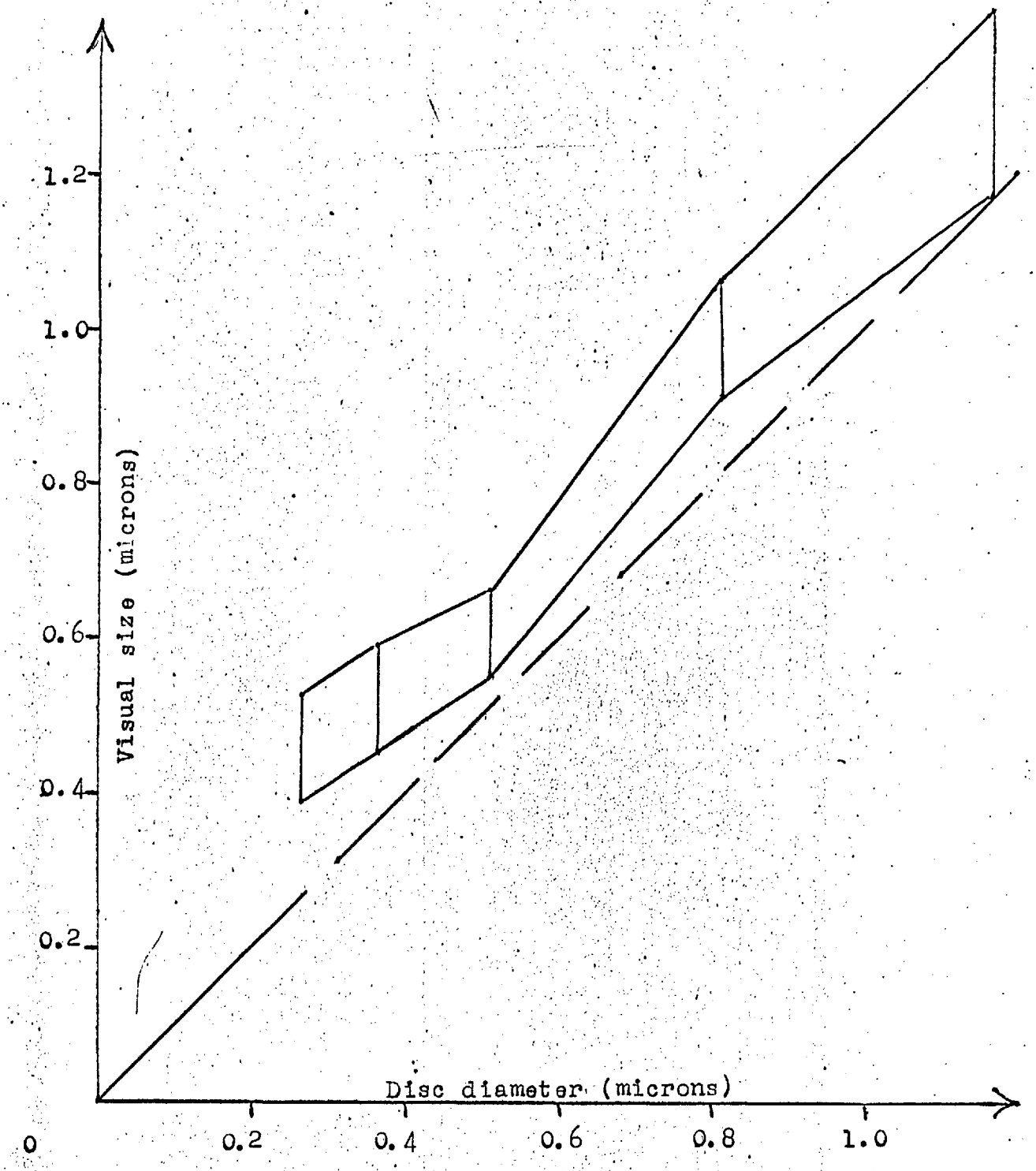


Fig.8.6. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. Mercury white light, objective N.A.=1.33, condenser N.A. = 0.37, magnification =1500X, screen luminance = 9 cd/m^2.

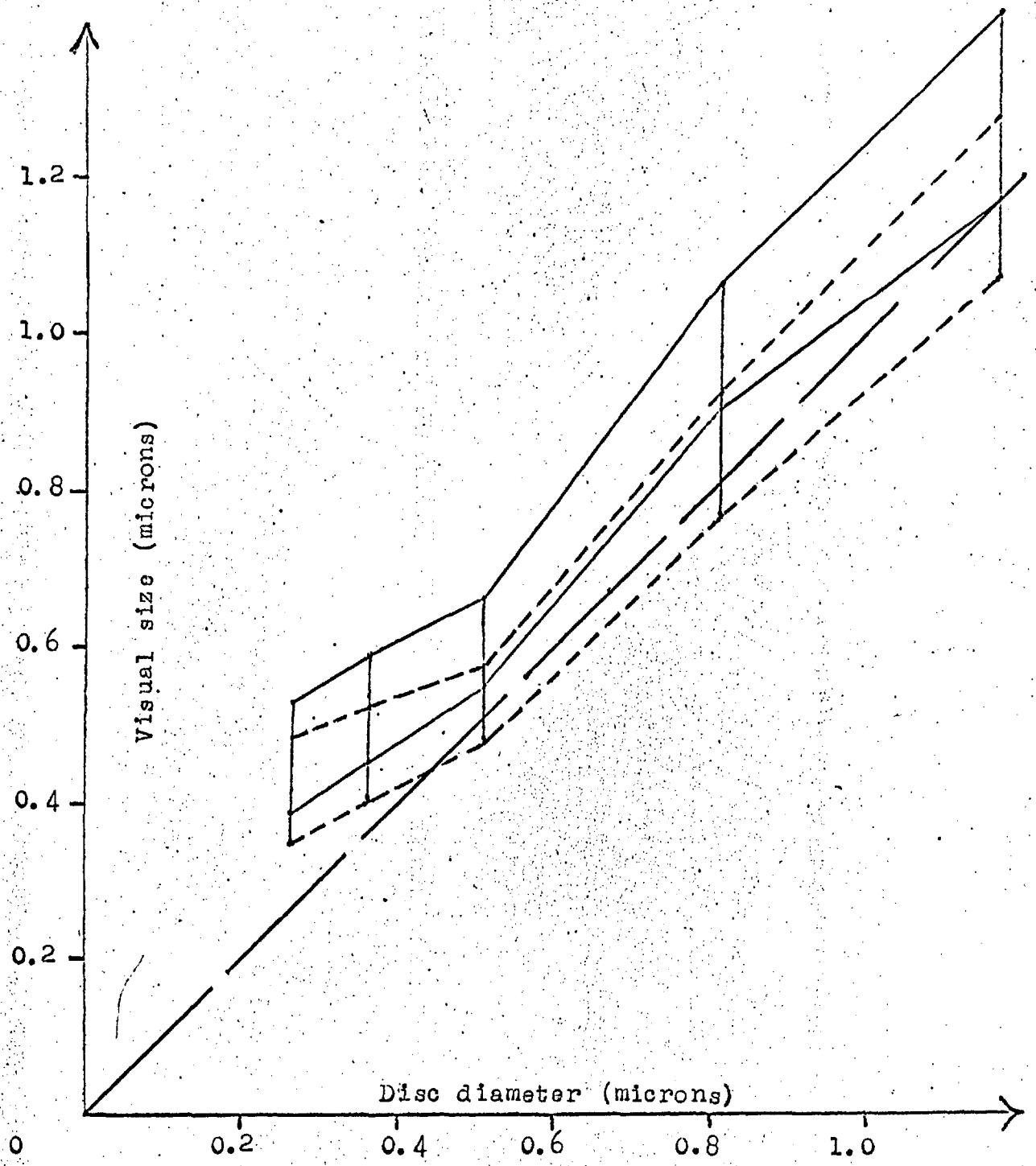


Fig.8.7. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. (a) Condenser N.A. = 0.37, (results from Fig 8.6. plotted with continuous line), (b) condenser N.A. = 1.27 (results from Fig.8.1. plotted with broken line),

the visual edge position moved steadily towards the lighter side of the true geometrical image of the edge as the condenser was stopped down.

The comparison made in Fig.8.7. was for visual sizes determined with different condenser N.A.'s and the same screen luminances. In practice of course if the condenser N.A. is reduced there is a reduction in the screen luminance the screen luminance being proportional to the square of the N.A. If when the condenser N.A. is decreased the effect of this decrease in screen luminance on the visual size is also considered it will be realized that whereas the effect of the former will be to increase the visual size of the opaque discs as illustrated in Fig.8.7. the effect of the latter will be to decrease it, as illustrated in Fig.8.5. The overall effect is illustrated in Fig.8.8. in which results with the condenser N.A. at 0.37 and the screen luminance at 9 candelas per square metre (i.e. from Fig. 8.6.) are compared with those with these quantities at 1.27 and 90 candelas per square metre respectively (i.e. from Fig.8.4.) the difference in screen luminance between the two cases being caused only by the change in condenser setting. In Fig.8.8. the results from Fig.8.6. are plotted with full lines and those from Fig.8.4. with broken lines. Comparison of the two shows that the effect on the visual size produced by the change in the condenser N.A. is slightly greater than that produced by the corresponding reduction in screen luminance, the overall effect being a

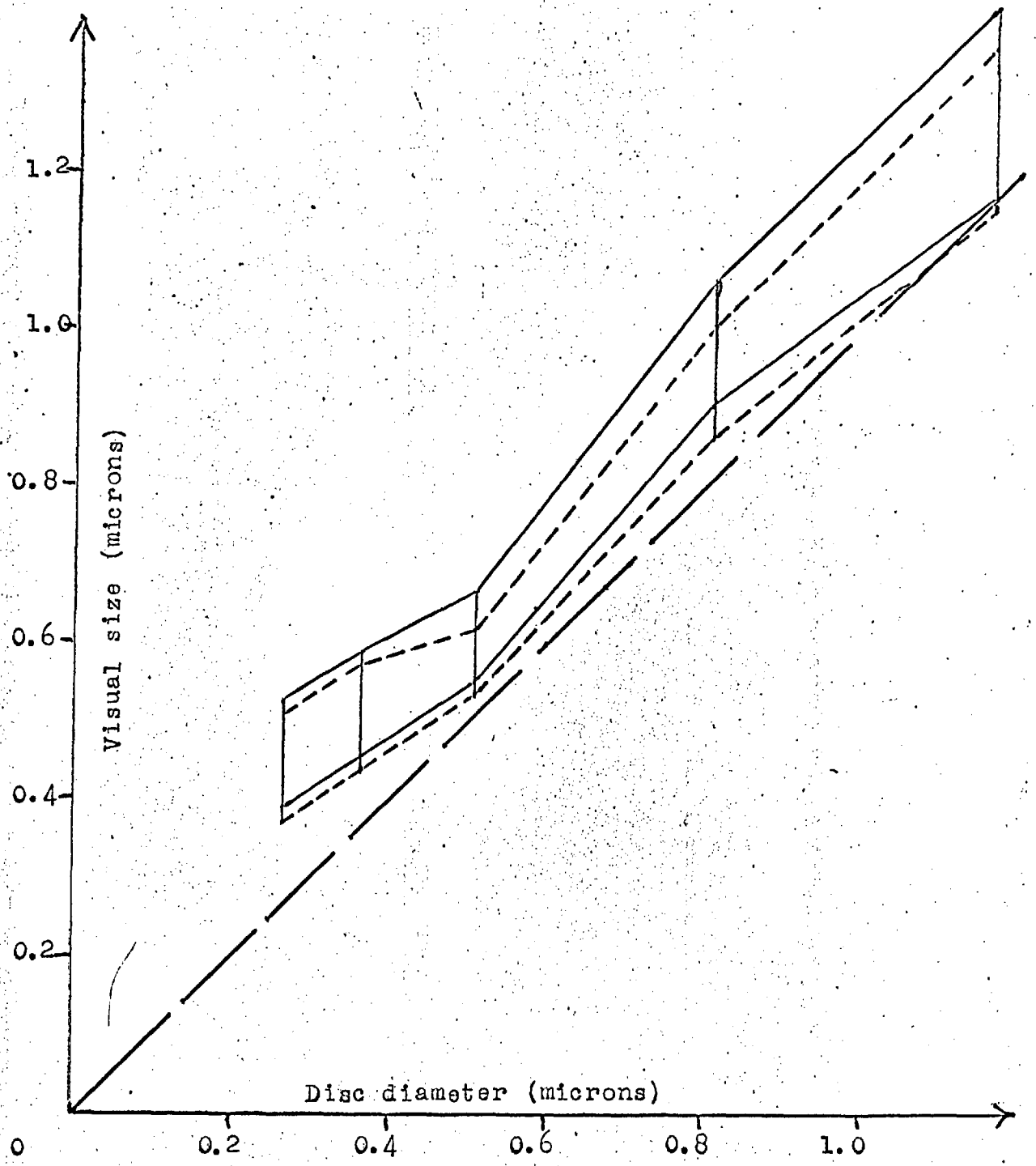


Fig.8.8. Variation of visual size with true diameter for opaque discs sized with the scanning block of the projection microscope. (a) Condenser N.A. = 0.37, screen luminance = 9 cd/m^2, (results from Fig.8.6. plotted with continuous line) (b) condenser N.A. = 1.27, screen luminance = 90cd/m^2, (results from Fig.8.4. plotted with broken line).

a slight increase in visual size over the whole size range, this increase being much less than that illustrated in Fig 8.7. due to the change in condenser N.A. alone.

Therefore the reduction in screen luminance which accompanies a reduction in condenser N.A. tends to compensate the increase in visual size produced by the latter, by causing a slightly smaller decrease in the visual size. This is of course only true for opaque objects in a bright surround. It is not true for bright objects for which both of the above effects reduce the visual size, as has been shown by Charman (1963(a) & (b)) with a direct microscope.

8.3. Results Using the Calibrated Wedge

In measuring the visual size of the opaque discs with the calibrated wedge, the visual edges used are again those of the dark disc, where the image brightness is just noticeably lower than that of its bright surround. The image is run in between the lines of the wedge until it just appears to touch both as illustrated in Fig.6.9. Both scales will thus show the same reading, which multiplied by a calibration factor gives the visual size.

Since with this method the visual size is given by a single scale reading it would be convenient if this were given directly in microns. The scale on the wedge as shown in Fig.6.9. is in centimetres. Therefore as the wedge ratio is of the order of 10:1, the necessary magnification for the wedge to be used as a direct reading instrument would

be of the order of 1000X. This can be achieved simply by using a 10X holoscopic eyepiece, in conjunction with the 90X Watson objective used previously. However with the objective used at its optimum tubelength and the eyepiece adjusted for zero lateral chromatic aberration with the white mercury light, the overall magnification on the screen was only of the order of 800X. In order to increase this it was necessary to increase the microscope tubelength above its optimum value, this being done with great precision in order to obtain exactly the right magnification necessary for direct reading.

The remaining conditions under which the first set of results were obtained with the calibrated wedge were as follows:-

the N.A. of the objective was 1.33 and that of the condenser 0.96, and the screen luminance was found to be 100 candelas per square metre. As usual 65 determinations were made for each different disc diameter, the results being summarized in Table 8 (v) and plotted in Fig.89. The 10X holoscopic eyepiece was replaced by the 20X one, thus giving an overall magnification of the order of 2000X. This meant that with the tubelength increased above its optimum value as before the calibration factor was $\frac{1}{2}$, only a very small adjustment of the tubelength being necessary to ensure this to be exactly so. As a result of the above change of the eyepiece, the screen luminance was now 25 candelas per square metre, all the other conditions being kept constant.

Table 8 (v) Visual sizing of opaque discs with the calibrated wedge of the projection microscope

10X holoscopic eyepiece.

Tubelength greater than optimum value for direct reading

Overall magnification = 1000X

Screen luminance = 100 cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	S μ
0.264	0.514	0.044
0.365	0.552	0.050
0.511	0.594	0.052
0.814	0.840	0.036
1.171	1.202	0.054

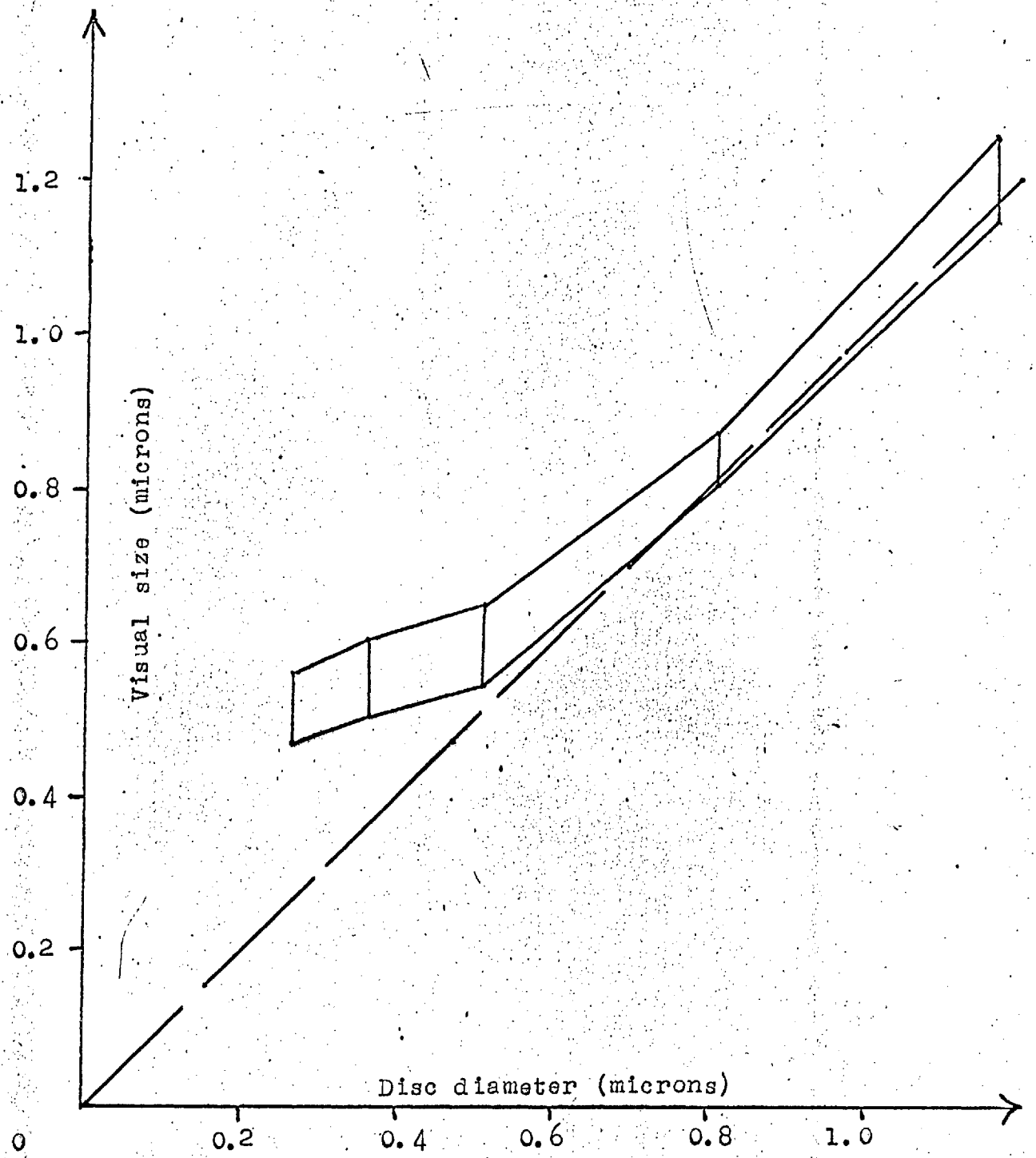


Fig.8.9. Variation of visual size with true diameter for opaque discs sized with the calibrated wedge of the projection microscope. Mercury white light, objective N.A.= 1.33, condenser N.A.= 0.96, magnification=1000X, screen luminance= 100 cd/m^2

With a calibration factor of $\frac{1}{2}$, the readings could be described as "semi-direct", and as before 65 determinations were made for each different disc diameter, the results being summarized in Table 8 (vi) and plotted in Fig.8.10.

In order to compare sizing results obtained using the calibrated wedge with those obtained using the scanning block given in the previous section it was first necessary to decrease the microscope tubelength down to its optimum value. This decreased the overall magnification to a value of the order of 1500X, which meant that the calibration factor was no longer simple, so that indirect readings would be taken. The above change in tubelength increased the screen luminance to 55 candelas per square metre, and all the remaining conditions were kept constant. 65 determinations were made for each disc diameter the results being summarized in Table 8 (vii) and plotted in Fig.8.11.

In Fig.8.12. these results are compared with a set obtained using the scanning block under conditions closest to those used above, i.e. the results from Fig.8.4. The conditions differ only in the value of the condenser setting an N.A. of 0.96 for the former and 1.27. for the latter - this also resulting in different screen luminances of 55 and 90 candelas per square metre respectivley. In Fig. 8.12. the results from Fig. 8.11 are plotted with full lines and those from Fig.8.4. with broken lines. Comparison of the two shows that the calibrated wedge gives consistently

Table 8 (vi) Visual sizing of opaque discs with the calibrated wedge of the projection microscope

20X holoscopic eyepiece

Tubelength greater than optimum value for "semi-direct" reading.

Overall magnification = 2000X

Screen luminance = 25 cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	s''' μ
0.264	0.401	0.065
0.365	0.443	0.063
0.511	0.502	0.061
0.814	0.795	0.077
1.171	1.101	0.065

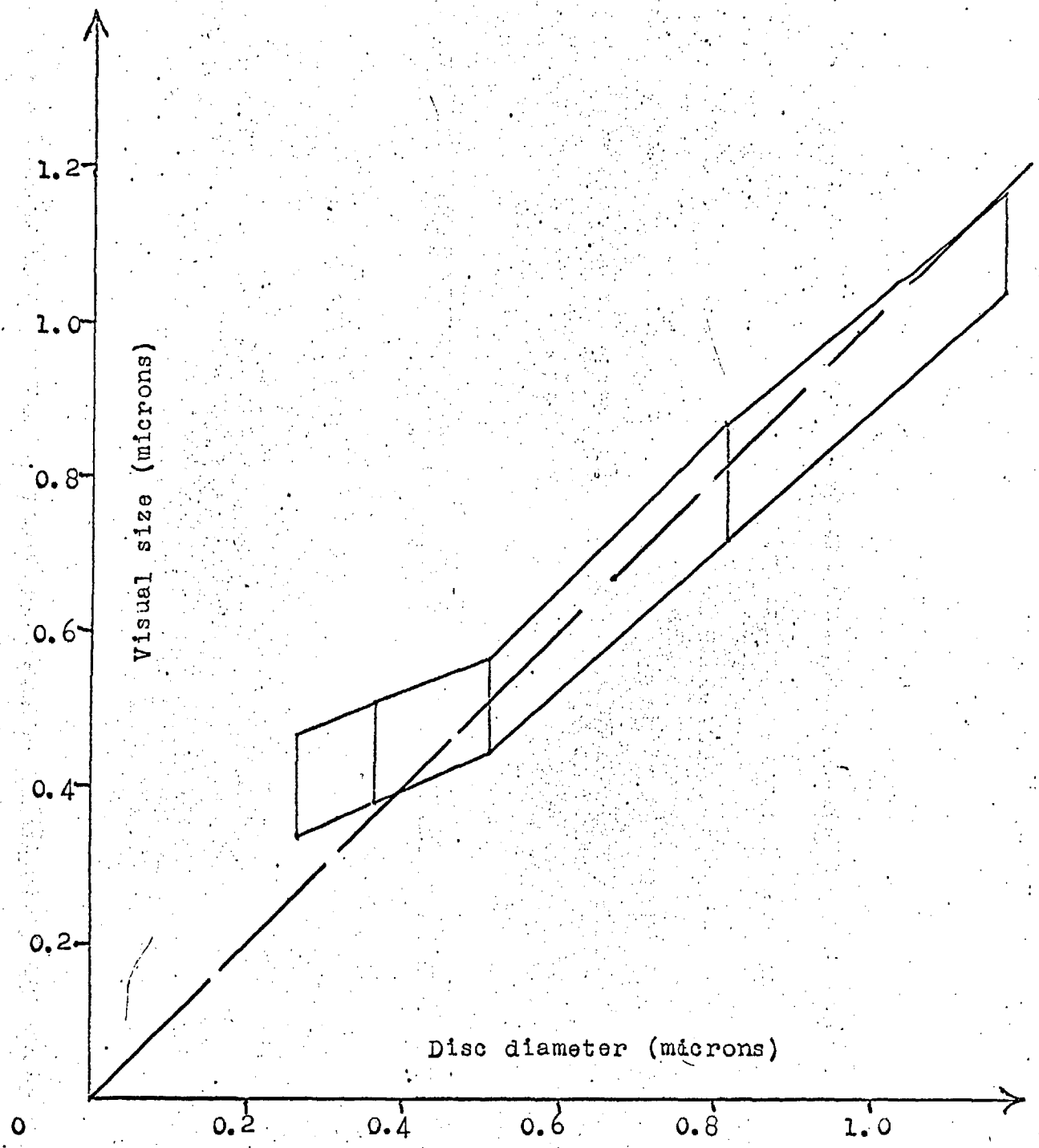


Fig.8.10. Variation of visual size with true diameter for opaque discs sized with the calibrated wedge of the projection microscope. Mercury white light, objective N.A. = 1.33, condenser N.A. = 0.96, magnification = 2000X, screen luminance = 25 cd/m^2.

Table 8 (vii) Visual sizing of opaque discs with the calibrated wedge of the projection microscope

20X holoscopic eyepiece

Tubelength at optimum value.

Overall magnification = 1500X

Screen luminance = 55 cd/m^2

$\bar{X}$ μ	$\bar{Z}$ μ	S'' μ
0.264	0.368	0.066
0.365	0.434	0.068
0.511	0.524	0.076
0.814	0.812	0.070
1.171	1.182	0.092

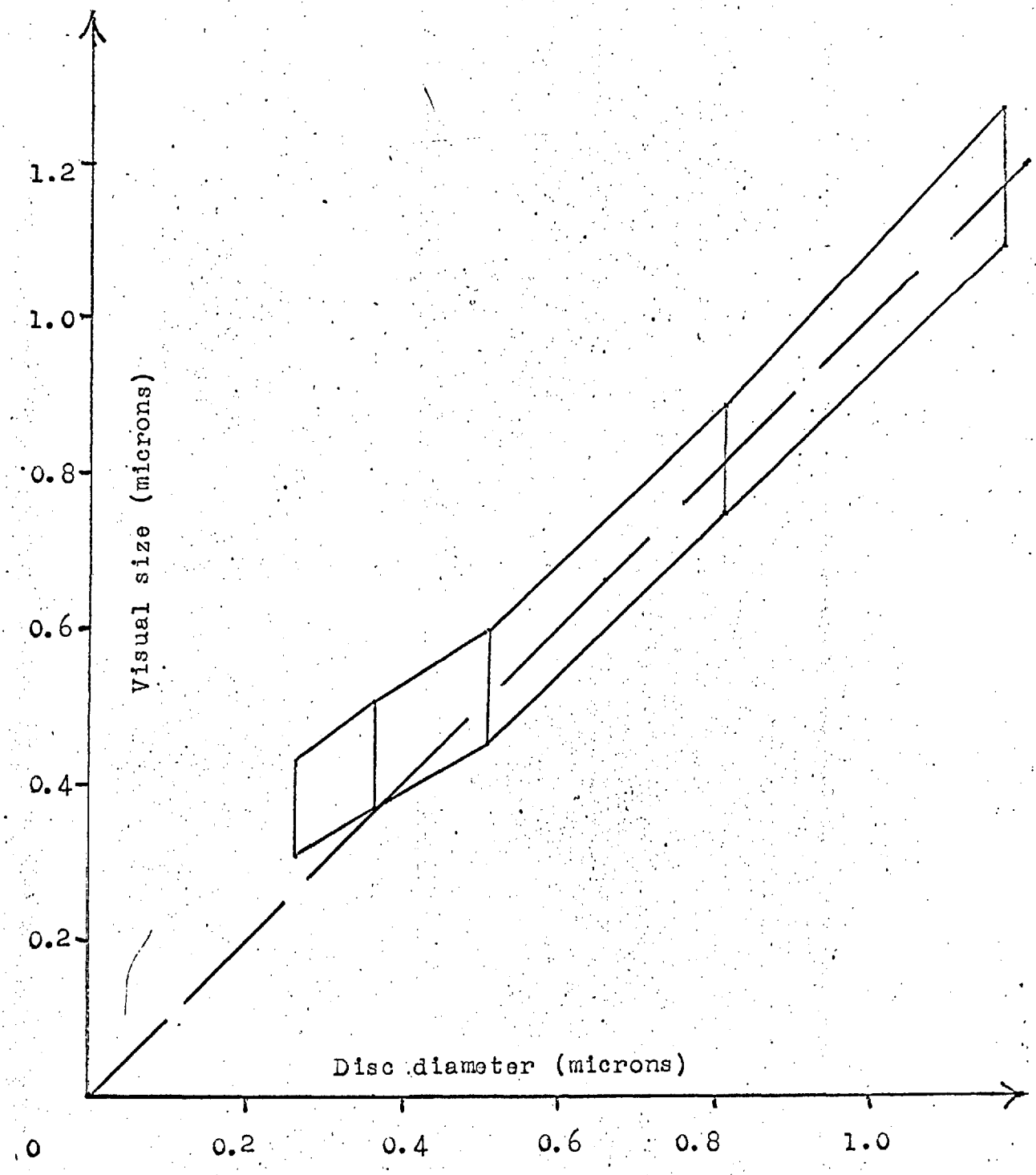


Fig.8.11. Variation of visual size with true diameter for opaque discs sized with the calibrated wedge of the projection microscope. Mercury white light, objective N.A. = 1.33, condenser N.A. = 0.96, magnification = 1500X, screen luminance = 55cd/m^2

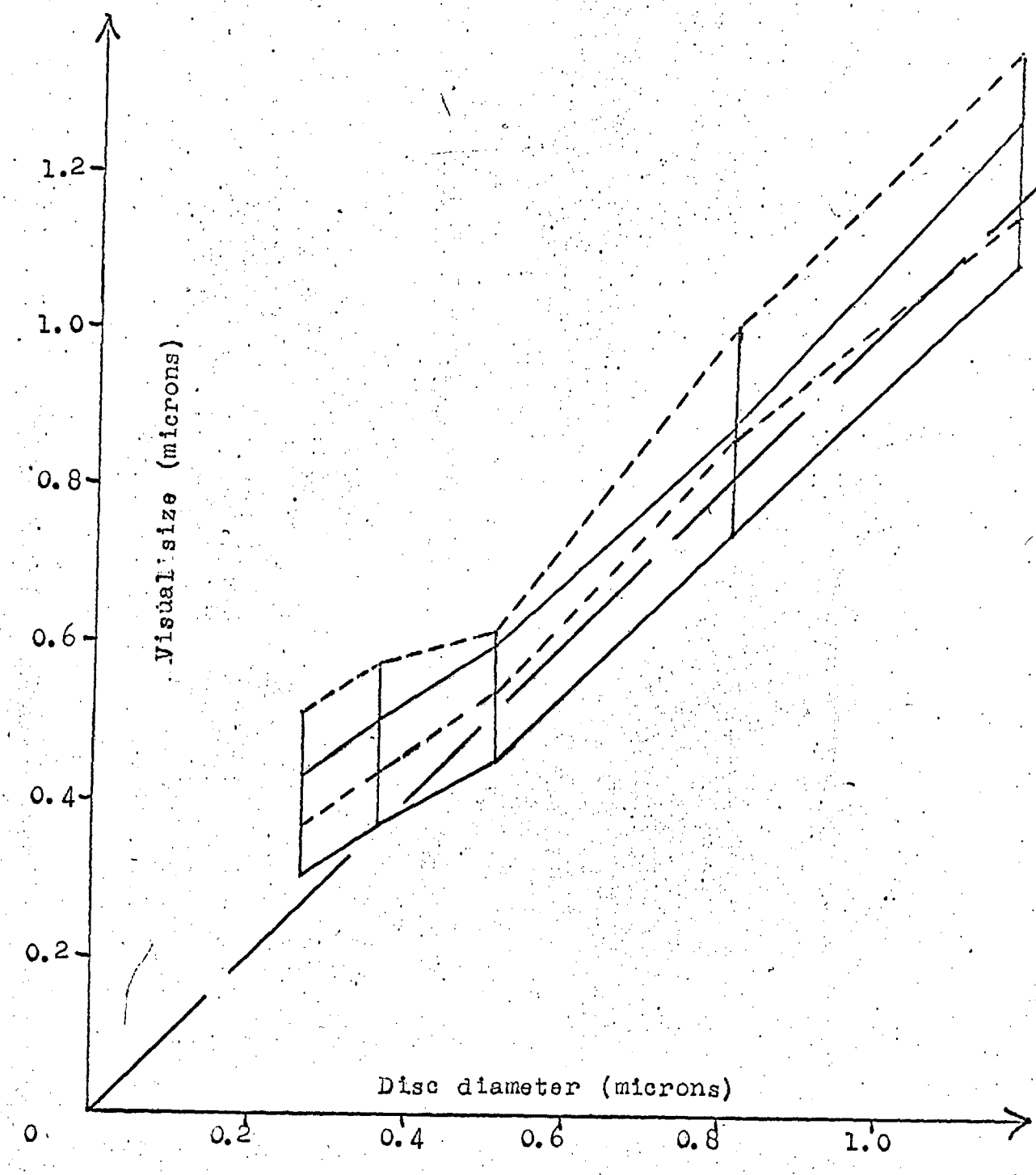


Fig.8.12. Variation of visual size with true diameter for opaque discs sized on the projection microscope with (a) the calibrated wedge (results from Fig 8.11. plotted with continuous line), (b) the scanning block (results from Fig.8.4. plotted with broken line).

smaller visual sizes over the whole range. This fact cannot be explained away by the slight difference in conditions. If the value of the condenser N.A. used in obtaining the scanning block results was reduced from 1.27 to 0.96, thereby also reducing the screen luminance from 90 to 55 candelas per square metre, the effect on the visual size can be judged by looking at Fig.8.8. This illustrates the effect on the visual size, using the scanning block, of reducing the condenser N.A. without compensating for the accompanying decrease in screen luminace. From Fig.8.8. it can be seen that a reduction of condenser N.A. from 1.27 to 0.37 produces a slight increase in visual size showing that a reduction from 1.27 to 0.96 would produce an increase in visual size which would be somewhat smaller than this. This means that for fair comparison in Fig.8.12. the scanning block results i.e. those plotted in broken lines should be increased very slightly thereby increasing the difference between the two sets of results plotted, showing that the slight difference in conditions is not responsible for this difference but in fact reduces it very slightly.

The reason for the difference in sizing results shown in Fig.8.12. lies in the two different sizing techniques used. With the calibrated wedge both edges of the opaque disc are set on the lines of the wedge simultaneously, so that the mean position that the eye fixes on during this

process is the centre of the opaque disc. With the scanning block however the two settings of the opaque disc on the vertical line are made separately so that the eye fixes only on the edges of the opaque disc. This means that with the calibrated wedge the effective field luminance will be less than that when using the scanning block. It is this reduction in effective field luminance with the calibrated wedge which produces the smaller visual sizes illustrated in Fig.8.12., similar to the smaller visual sizes produced by an actual reduction in screen luminance illustrated in Fig.8.5.

Now although the scanning block and calibrated wedge produce visual sizing results which have different systematic errors as discussed above, it can be seen from Fig.8.12. that the standard deviations i.e. the random errors of the visual sizes produced by the two systems are very similar. This suggests that the systems are equally sensitive i.e. have the same setting precision. This is only to be expected since both systems use the same criterion for assessing the position of the visual edge (i.e. where the image brightness is just noticeably lower than that of its bright surround in the case of the opaque disc,) and both systems locate the position of this visual edge by setting it on straight black lines. Therefore to this extent the two systems are similar, thus producing similar setting precisions, but beyond this similarity differences between

the two systems occur, these differences being responsible for the different systematic errors as described above.

All the results plotted in this chapter i.e. in Figs. 8.1. to 8.12. confirm yet again the facts that below an object diameter of about 0.4 μ (i.e. approx 1.5. Airy units) the visual size tends to a constant value dependent on the optical and visual conditons, whereas above this value of object diameter the visual size is fairly consistently in excess of the true size by an amount which is also dependent on the prevailing conditions.

In Fig. 8.12. is illustrated the fact that this excess of visual size over true size is different for the two sizing systems considered in this chapter as discussed above. In particular allowing for the slight difference in optical and visual conditions prevailing the difference between these two excesses (i.e. the difference between the two systematic errors - this difference actually being consistent over the whole size range, unlike the systematic errors themselves) is equal to about 0.09 μ (i.e. approx 0.4 Airy Units).

CHAPTER 9

DISCUSSION AND CONCLUSIONS

9.1. Introduction

From the experimental results given in the previous chapters it is possible to judge the influence of the viewing and sizing systems on size determination by visual microscopy, and in addition to consider the possible influence of the observer himself. However before doing so it is first necessary to comment a little further on the test objects which were used in all the visual sizing experiments i.e. the small opaque discs.

9.2. The Opaque Disc Test Objects

It is apparent from the scanning results given in Fig.4.5. that quantitative differences exist between the form of the images of the opaque discs predicted by scalar diffraction theory and those found in practice with a high aperture objective. Although the optical and recording conditions used experimentally might be blamed in part for these discrepancies, any that occur over and above the limits of experimental error are due to either the fact that with a thickness of about 0.08μ the objects cannot be considered as negligibly thin or to the slight aberrations in the microscope objective or to the approximations of scalar diffraction theory being inapplicable to systems of high N.A.

In Fig.4.5. divergence between theory and experiment outside the limits of experimental error occurs only at the lower end of the size range, where one would expect the thickness of the object ot influence the expàrimental results, if it were going to at all. H owever Charman (1963(d)) has shown that doubling the thickness of the evaporated metal films used in preparing the test objects appeared to introduce no significant changes in the image profiles that would indicate that this factor was of paramount importance.

The effect of slight aberrations in a microscope objective working at high N.A. on the diffraction images of circular apertures in an opaque film, has been investigated by Watrasiewicz (1965(b)). He showed that while improved aberration correction of an objective improved agreement with theory there were still some residual deviations particularly for smaller apertures.

Further work by Watrasiewicz (1965(b) and 1965(c)) has shown that these residual deviations are due to the breakdown of scalar theory at high numerical apertures. This is in agreement with the calculations of Richards and Wolf (1956 and 1959) based on the electromagnetic theory who have shown that the simple point image formed by a converging spherical wave of large numerical aperture is markedly different from the Airy disc.

Thus any difference between images formed by microscope objectives with large numerical aperture and theoretical predictions based on scalar theory are only to be expected. However as has been suggested by Martin (1966) these theoretical predictions may be considered as a general guide in microscopy near the resolution limit, this fact providing a fitting description to the results plotted in Fig. 4.5.

9.3. The Influence of the Viewing System

In section 2.2. the relative merits of direct and projection microscope viewing systems were considered, and although projection viewing was preferred due to its improved comfort and convenience, two reasons were given suggesting that the performance of the projection microscope would be relatively poor. The first was the flatness of the image. However in this investigation the thickness of the objects used was about 0.08 μ and although this cannot be considered as negligibly thin it is still much less than the depth of focus of the microscope objective used (($\sim$0.35 μ), and therefore the fact that the screen image is flat is not of great importance. The second reason was the grain of the projection screen. However in this investigation the screen that was used was grainless, its development and construction having been described in Chapter 5 and therefore this objection to projection viewing was completely overcome. Both objections to projection viewing having been overcome for this investigation, it is possible to

compare the visual sizing results obtained using both systems under the same optical and visual conditions with the same sizing system i.e. the scanning block and single vertical line graticule described in Chapter 6.

In comparing results obtained by direct and projection viewing it is first necessary to note that the field luminances were measured differently. With direct viewing the quantity that was determined was the level of retinal illumination in trolands, 1 troland being the retinal illumination produced by a surface having a luminance of one candela per square metre when the pupil area is one square millimetre. But with projection viewing the actual screen luminances were measured in candelas per square metre, values of 9, 18 and 90 cd/m^2 being used. Taking into account the decrease in pupil size with increasing screen luminance and also the limited Stiles-Crawford effect which lessens the contribution to the level of retinal illumination of the outer parts of the eye lens, it can be shown that the above three screen luminances produce retinal illuminations of 10^2, 1.5×10^2 7×10^2 trolands respectively. These values coincide fairly well with two of the values of retinal illumination used in direct viewing i.e. 10^2 and 10^3 trolands.

Therefore it is now reasonable to compare results, starting with those from Figs.7.1. and 8.1. The conditions under which both these sets of results were obtained were

as follows :-

The N.A. of the objective was 1.33 and that of the condenser 1.27. The retinal illumination was 10^2 trolands (having been produced in the projection case by a screen luminance of 9 cd/m^2) . The magnifications were very slightly different, being 1800X and 1500X for direct and projection viewing respectively The only important difference in the two sets of conditions was in the spectral composition of the light used. In the projection case white unfiltered mercury light was used, wheras with the direct microscope the mercury lamp was used in conjunction with a green filter which transmitted only light of wavelength $\lambda = 0.546\mu$.

In Fig.9.1. results from Fig.7.1. (direct) plotted in broken lines, and Fig.8.1. (projection) plotted in full lines are compared. From Fig.9.1. it can be seen that there is virtually no systematic difference between the two sets of results, although random differences occur in differing directions along the size range.

To check whether or not the spectral difference was a complicating factor in Fig.9.1. the results from Fig.7.1 are compared with those from Fig.8.2. which were obtained with projection viewing, using the green filter with the mercury lamp, but with a retinal illumination of 1.5×10^2 trolands (produced by a screen luminance of 18 cd/m^2). Therefore although these two sets of results were obtained with light of identical spectral composition the retinal

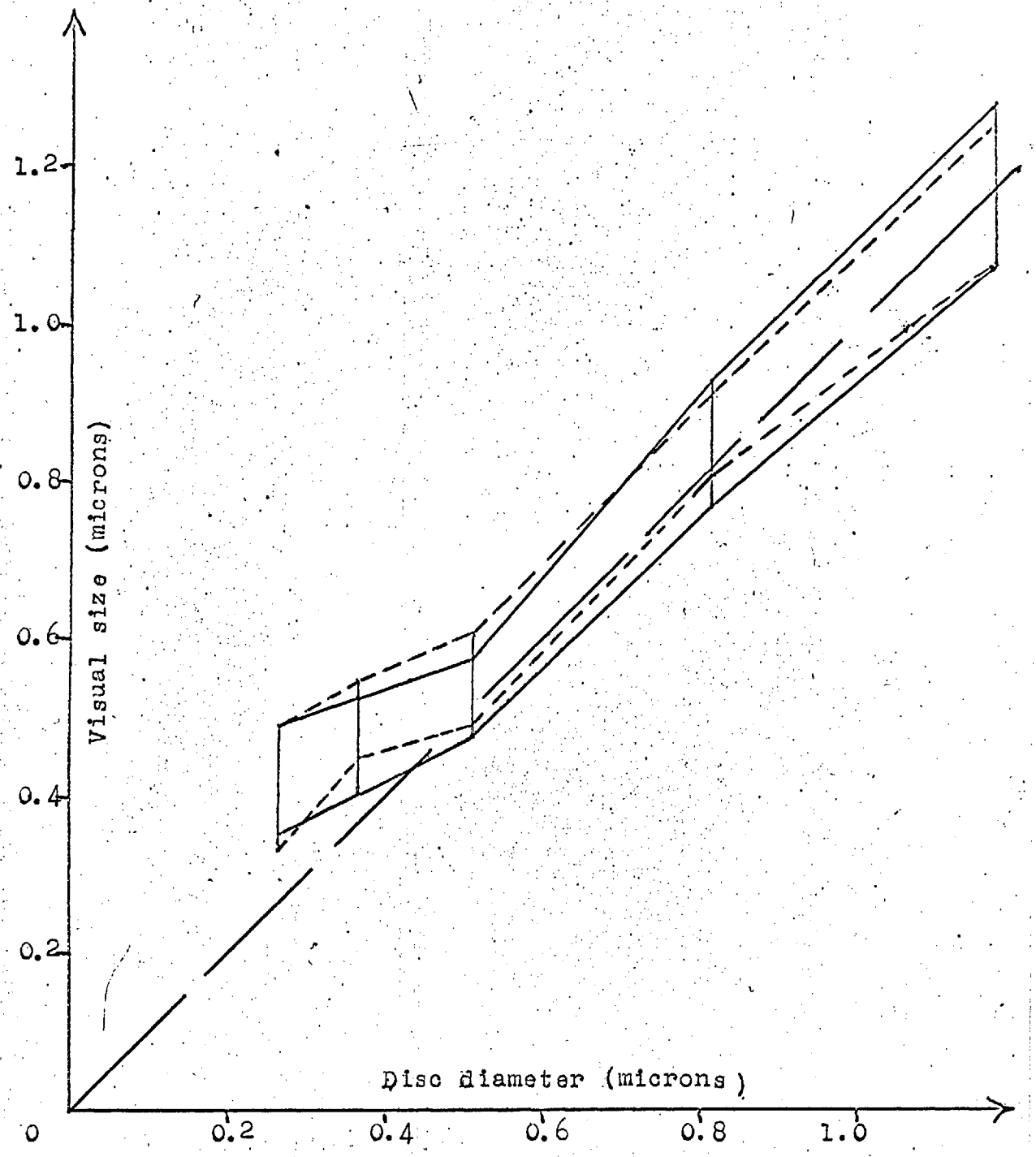


Fig. 9.1. Variation of visual size with true diameter for opaque discs sized with the scanning block of (a) the direct microscope with mercury green light (results from Fig. 7.1. plotted with broken line), (b) the projection microscope with mercury white light (results from Fig. 8.1. plotted with continuous line).

illuminations used now differ by a factor of two-thirds.

In Fig.9.2. results from Fig.7.1. (direct) plotted in broken lines and Fig.8.2. (projection) plotted in full lines are compared. From Fig.9.2. it can be seen that once again there is very little difference between the two sets of results thus showing that the difference in the spectral composition of the light used occuring in Fig.9.1 was not a complicating factor in comparing the two sets of results shown therein. This is not surprising since this spectral difference was shown to have a negligible effect in Chapter 8 as illustrated in Fig.8.3.

A further comparison on results obtained from the two different viewing systems under similar conditions may be made by using the results from Figs.7.2 and 8.4. Those from Fig.7.2. (direct) were obtained with a retinal illumination of 10^3 trolands and green mercury light, and those from Fig.8.4. (projection) with a retinal illumination of 7×10^2 trolands (produced by a screen luminance of 90 cd/m^2) and white mercury light. They are compared in the usual way, in Fig.9.3. from which it can be seen that once again there is no uniform systematic difference between them. However random differences between them now show up as greater values for the visual size by projection viewing at the upper end of the size range, and smaller values at the lower end. This trend can be seen, but to a lesser extent in Fig.9.2. and also in Fig.9.1. to an

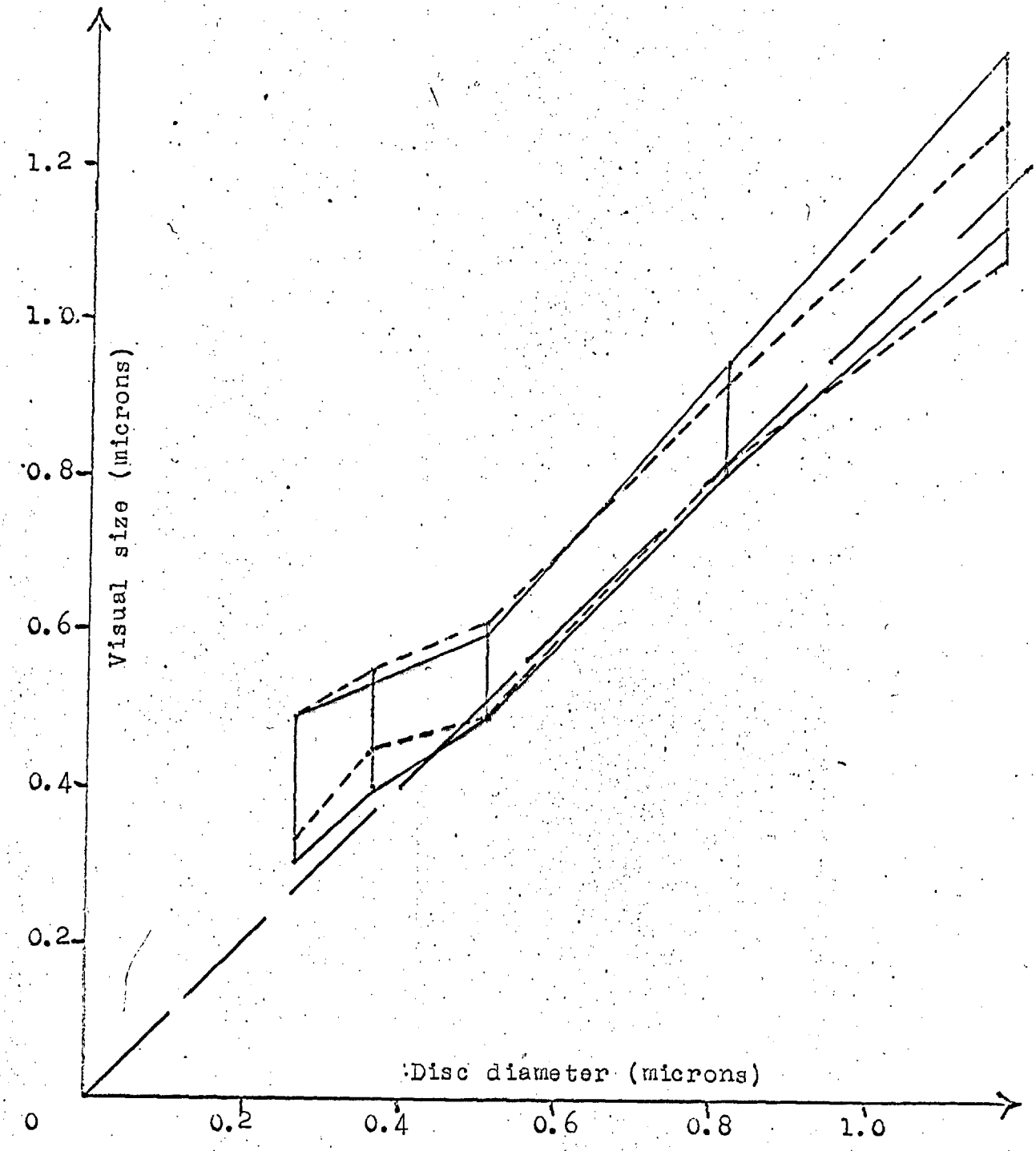


Fig.9.2. Variation of visual size with true diameter for opaque discs sized with the scanning block of (a) the direct microscope with a retinal illumination of 10^2 trolands (results from Fig.7.1. plotted with broken line), (b) the projection microscope with a retinal illumination of 1.5×10^2 trolands (results from Fig.8.2. plotted with continuous line).

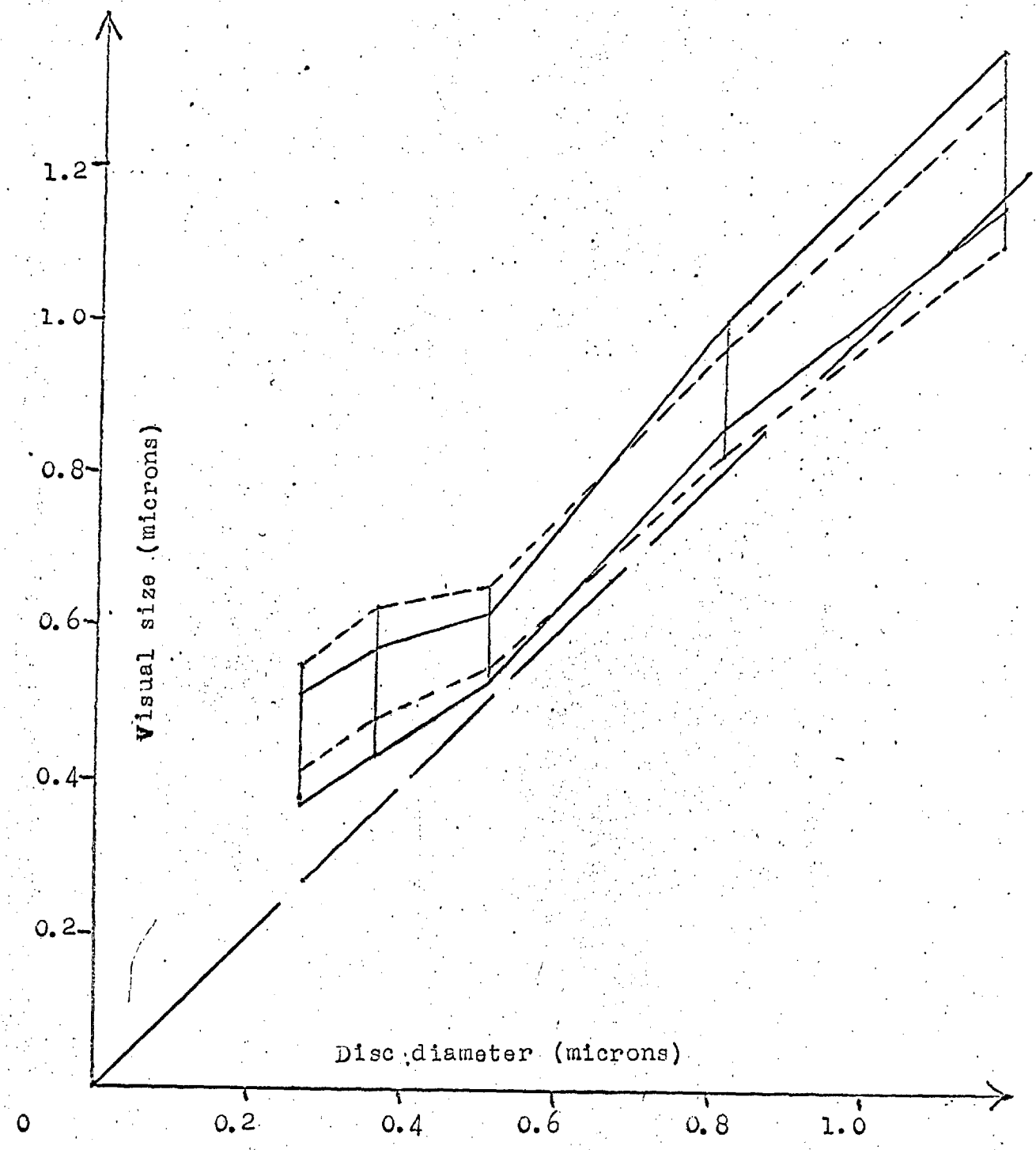


Fig.9.3. Variation of visual size with true diameter for opaque discs sized with the scanning block of (a) the direct microscope with mercury green light and a retinal illumination of 10^3 trolands (results from Fig.7.2. plotted with broken line), (b) the prpjection microscope with mercury white light and a retinal illumination of 7×10^2 trolands (results from Fig.8.4. plotted with continuous line.

even smaller extent, although in all three cases when considering the size range as a whole, the results obtained by the two methods are almost identical, this being so for the standard deviations as well as the results themselves. Therefore visual sizing by direct or projection microscopy will produce values having identical systematic and random errors. This fact was suggested to be true by Charman (1961) but now has been firmly established experimentally.

The above now means that provided only 'thin' objects and a grainless screen are used, the advantages of comfort and convenience obtained in projection viewing may be fully utilized without fear of there being any adverse effect on the visual sizing results.

9.4. The Influence of the Sizing System

In Section 2.3. the relative merits of the moving crosswire and sheared image methods of sizing were discussed. The important differences between the two methods may be summarized as follows:-

(1) Different objects are set on the visual edge, namely the crosswire and the second image.

(2) Different criteria are used for assessing the position of the visual edge with the above objects. The crosswire is set at the point where the subjective brightness of the image just differs from that of the surroundingfield whereas the second image is set to overlap the first, such that the

subjective brightness at the point of overlap is equal to that at the centre of each image.

(3) The setting error of the sheared image method (approx. $\pm$ 0.10 Airy units) is less than half that of the crosswire method (approx. $\pm$0.25 Airy units).

No comparison can be made between the systematic errors of the two methods, for although that of the crosswire method is known to be of the order of plus half an Airy unit, the exact value depending upon the prevailing conditions, that of the sheared image method has not been determined by experiment.

The small setting error of the sheared image method can be attributed to the fact that the overlapping of the edges of the two images produces an increase in the variation of the subjective brightness with movement, thus making the method more sensitive.

In section 7.3. the experimental results obtained with the moving crosswire of a filar micrometer eyepiece were compared with those obtained with a scanning block which moved the image across a stationary crosswire. All that distinguishes the two methods from one another is the fact that with the former the image is stationary and the crosswire moves while with the latter the crosswire is stationary and the image moves. Apart from this everything else about the two methods is identical i.e. the same object for setting on the visual edge and the same criterion

for assessing the position of the visual edge. Results obtained by both methods were compared in Fig.7.7. from which it was observed that after allowing for the slight difference in the prevailing levels of retinal illumination, the systematic error was the same for both methods i.e. 0.5 Airy units, showing that it is unaffected by whether the crosswire or the image moves. However it was found that the setting error of the scanning block method was slightly larger (approx. $\pm$0.30 Airy units).

In the last section it was shown that the scanning block method produced the same results, when used with a stationary crosswire (a single vertical line graticule) for direct viewing as when used with a single vertical line drawn on a screen for projection viewing this applying to both systematic and random(setting)errors. Therfore the comparison made between the scanning block and calibrated wedge mthods of sizing opaque discs with projection viewing in Section 8.3. is in fact a comparison between the latter and single line setting methods as a whole. This comparison showed that

(1) The same objects are set on the visual edge, namely black straight lines.

(2) With the scanning block these settings are made separately but with the calibrated wedge they are made simultaneously. So that while with the former the eye remains fixed on the edge of the image with the latter the

mean position that the eye fixes on is the centre of the image.

(3) The same criterion is used for assessing the position of the visual edge.

Also from Fig. 8.12 it was observed that

(4) The two methods have the same setting error (approx. $\pm$ 0.30 Airy units)

(5) The systematic error of the scanning block method exceeds that of the calibrated wedge method by approximately 0.4 Airy units over the whole size range. The systematic error of the latter is therefore of the order of plus a tenth of an Airy unit, the exact value again depending upon the prevailing conditions.

On examining the above five points, it is fairly obvious that the cause of the different systematic errors mentioned in point (5) must be the different fixation positions of the eye mentioned in point (2). The latter producing different effective field luminances which produce the different systematic errors as already discussed in Section 8.3.

Considering then the whole of this section so far, it is fair to say that there are three important factors in any one sizing system which will influence its sizing errors:-

(1) The object used to set on the visual edge - this will influence the random error of the system i.e. its sensitivity or precision.

(2) The mean position of fixation of the eye during the edge setting process - this will influence the systematic error i.e. its oversize (or undersize).

(3) The criterion used for assessing the position of the visual edge with the object in question - this will influence the systematic error and possibly the random error as well.

Considering the above three factors it is now possible to estimate the errorsinvolved in a system of visual sizing as yet not mentioned in this chapter so far, but described in Section 1.12, namely the"globe and circle" graticule. This is of course used only to classify a particulate sample into a limited number of size groups and with the opaque disc test objects would consist of a series of opaque discs of standard sizes for comparison. For this method the above mentioned three factors are as follows:-

(1) No object is used to set on the visual edge, a particular opaque disc is judged to have a visual size above or below a particular "globe" by direct comparison.

(2) The mean position of fixation of the eye during a comparison will be the centre of the opaque disc- as was the case with the calibrated wedge method.

(3) The criterion used for assessing the position of the visual edge during a comparison is the same as that used in the line setting methods i.e. the point where the subjective brightness of the image just differs (is just darker for an opaque disc) from that of the surrounding (bright) field - in particular the same as was used for the

calibrated wedge method.

It can be seen now that the two factors affecting the systematic error (i.e. (2) & (3)) are identical for the globe and circle and calibrated wedge. It is reasonable to assume therefore that these two systems will have the same systematic error i.e. of the order of $\pm$ 0.1. Airy units. This means that there is only very slight oversizing with the globe and circle method, a fact noted by Heywood (1945). As this is a size grouping method it simply means that the edge of each size range i.e. the range of sizes of the 'globes' are overestimated by $\pm$ 0.1. Airy units.

Now the two factors which effect the random error of the method are (1) and (3). Now (3) as mentioned previously is the same as for the calibrated wedge, but (1) is completely different, and therefore it would be impossible even to estimate the random error for the globe and circle method. However as this is a size grouping method in which a large number of particles must always be examined the random error is not nearly as important here, as in the previous methods, where one might possibly want to determine the exact size of one particular particle with one single determination. Therefor the fact that the random error of the globe and circle method cannot be estimated here is of no real importance; not as in the case of the systematic error of the sheared image method, the experimental determination of which for the opaque discs would clearly be

helpful in any further consideration of that sizing method,.

9.5. The Influence of the Observer

In order to check that sizing results obtained by another observer would not differ from those obtained by the author the results plotted in Fig.7.6. i.e. those obtained with the filar micrometer eyepiece with a moving crosswire are compared with a set obtained by Charman (1963(b)) using the same sizing system under identical conditions and within the same size range of opaque discs.

The comparison is made in Fig.9.4. with the author's results from Fig.7.6. plotted in continuous lines and Charman's (1963(b)) plotted in broken lines. It shows that over the whole range of overlap of disc diameter, both sets show very good agreement. This suggests that all the sizing results reported in this thesis could be taken as typical for any trained observer.

9.6. Conclusions

The conclusions made from the experiments described in this thesis are set out in Table 9 (i) and constitute the systematic and random errors of all the sizing and viewing systems considered. The following supplementary comments however are necessary:-

Whereas the value of the random errors have been found to be fairly consistent, the same has only been found to be true for the values of the systematic errors when (a) the value of the opaque disc diameter exceeds 1.5. Airy units.

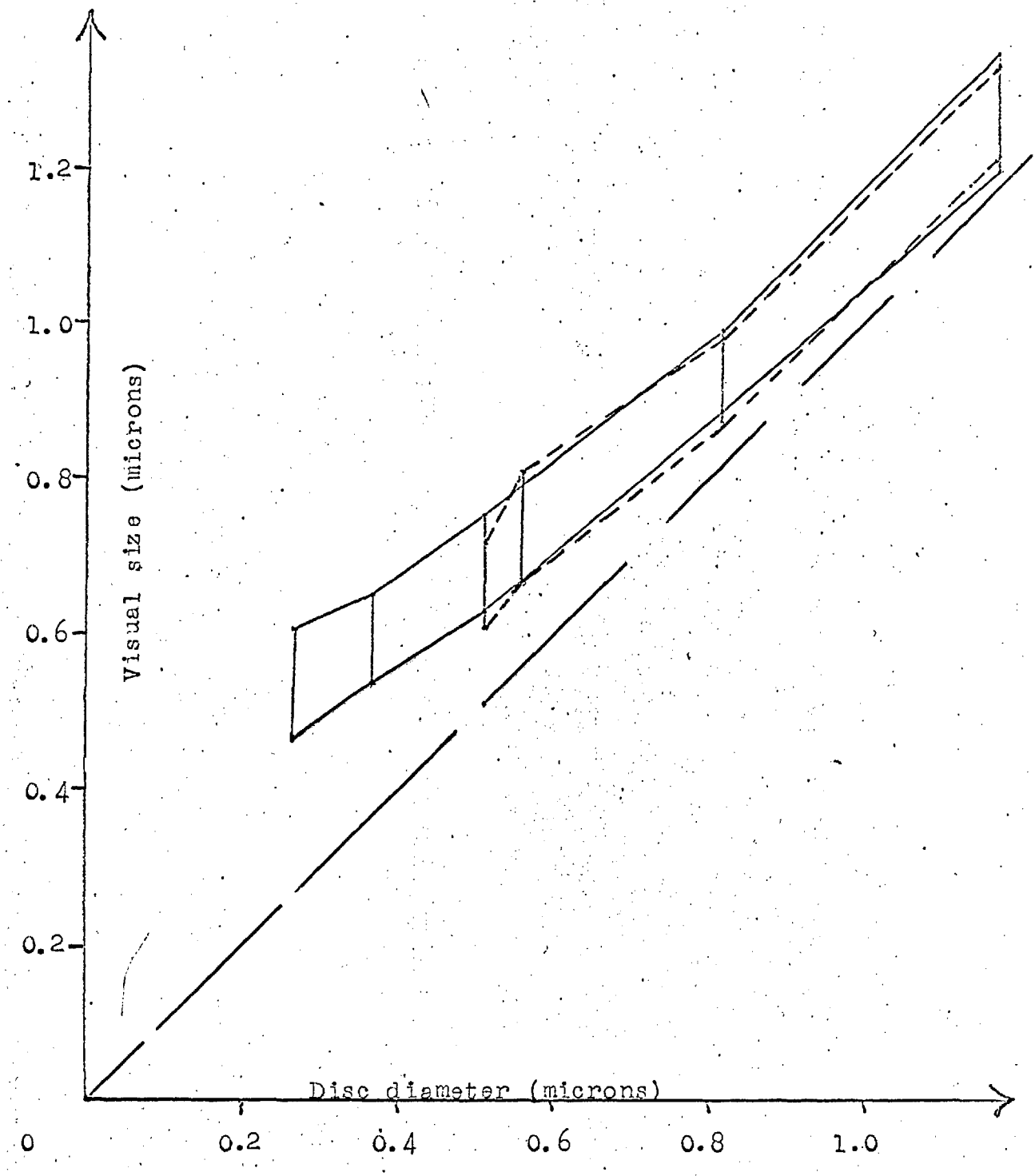


Fig.9.4. Variation of visual size with true diameter for opaque discs sized with a filar micrometer eyepiece, by (a) the author (results from Fig.7.6. plotted in continuous line), (b) Charman (plotted in broken line).

Table 9 (i) The influence of the viewing and sizing systems on the visual sizes of opaque discs which are thin and of high contrast.

System	Systematic Error* (oversize)	Random Error (setting error)
	(Airy units)	
Sheared Image	?	± 0.10
Filar Micrometer Eyepiece with Moving Crosswire	+ 0.5	± 0.25
Scanning Block with Stationary Crosswire for Direct Viewing	+ 0.5	± 0.30
Scanning Block with Line Ruled on Screen for Projection Viewing	+ 0.5	± 0.30
Calibrated Wedge	+ 0.1	± 0.30
Globe and Circle Graticule	+ 0.1 (estimated)	- -

* Subject to limitations of disc diameter and working conditions (see section 9.6.)

(b) the prevailing working conditions were as specified by Charman (1963(a) & (b)), (i.e. a minimum magnification of 150X the objective N.A., a comfortably bright image field etc.) So that while the random errors are always as given the systematic errors are only as given subject to limitations on the disc diameter and the prevailing working conditions as indicated in Table 9 (i).

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USE OF AN AROCLOR RESIN AS HIGH REFRACTIVE INDEX MOUNTING MEDIUM FOR MICROSCOPY

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IN order to survey the concentration of coal and stone dust in the air of mines, samples are collected as a deposit on a microscope cover-glass. In normal practice the cover-glass is then dry-mounted and counts of the particles in several size groups ranging from 0·5 μ to 5 μ are made, using an eyepiece graticule with the magnification calibration by means of a stage micrometer in the usual way. In order to achieve maximum resolution an oil-immersion objective of N.A. about 1·3 is used.

It appeared to us when investigating visual sizing and counting techniques for the National Coal Board that there would be many advantages in using a mounting medium instead of dry-mounting: (*a*) the resolution would be increased, since the full numerical aperture of the objective could be used, whereas with a dry slide the maximum effective N.A. is 1·0; (*b*) the depth of focus is greater when using an immersion medium for the same N.A., for the focal range $\delta\zeta$ is given by the expression:

$$\delta\zeta = \pm \frac{\lambda}{8n \sin^2 \frac{1}{2}U} \quad . \quad . \quad . \quad . \quad (1)$$

where λ is the wavelength of the light, U is the semi-angle of the cone of light collected by the objective and n is the refractive index of the immersion medium; (*c*) the luminance of the image is increased according to the square of the N.A., a gain which could be of importance in projection work; (*d*) the air path in the dry slide introduces positive or under-corrected spherical aberration, which gives a less well-defined image. In addition to these optical advantages there is the practical advantage that a suitable mounting medium would provide a permanent preparation, whereas in dry-mounted thermal-precipitator-dust slides the particles are held to the cover-glass by very weak forces and are easily dislodged by slight shocks.

A suitable mounting medium would be quick and simple to use, i.e. not requiring prolonged baking, and it would have a rather high refractive index, say about 1·65, so that transparent stone particles, which are mostly quartz (refractive index 1·54) would be visible. Some proprietary mounting media of high refractive index, e.g. "Sirax", which used to be available are now apparently no longer made. We discussed this problem with Dr. E. Bovey of B.S.I.R.A. and he suggested trying Aroclor 4465, an epoxy resin made by Monsanto Chemicals, Ltd. It turns out that this had indeed the right combination of properties. It is a clear resinous material, yellow in bulk but colourless in the thickness used in mounting. It melts at about 90°C fairly sharply and wets glass, coal and stone dust

well. We measured its refractive indices on a small prism of about 10° angle which we cast between glass plates; the results are given in Table I below:

TABLE I

REFRACTIVE INDICES OF AROCLOR 4465

Wavelength, Ångstroms	4046	4800	5790	6563
Refractive index	1·7155	1·6859	1·6665	1·6585

Refractive index values corrected to ±0·0005.

We found that a convenient way to mount dust slides in Aroclor was to place the cover-glass gently on a clean slide, the dust deposit being on the under side of the cover-glass; a piece of Aroclor a few cubic millimetres in size was then placed on the slide touching the edge of the cover-slip and the whole raised to a temperature of about 110°C in an oven; in a few minutes the Aroclor melted and was drawn into the space between the slide and the cover-glass; the preparation could then be removed from the oven and was complete. It was found that the thickness of the Aroclor film so formed was consistently between 10 and 15 microns. Slides so produced seem to be quite permanent.

If the flow of the Aroclor across the slide is stopped halfway by taking it out of the oven a comparison can be made on the same slide of the viewing conditions. It is found that the depth of focus is increased and the field of view is brighter, as predicted. There is an appreciable gain in crispness and brilliance over the air-mounted portion and stone particles can still be quite clearly distinguished.

We are grateful to Dr. E. Bovey for advice about mounting media, to Mr. J. M. Campbell of Monsanto Chemicals, Ltd., for a gift of 100 g of Aroclor 4465, and to the National Coal Board for a grant in aid of this work. The opinions expressed are those of the authors and not necessarily those of the Board.

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Grainless Screens for Projection Microscopy

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Back-projection screens for projection microscopy with high luminance and no loss of definition are described; the screen grain is removed by the slow relative movement of two ground-glass screens placed face to face.

IN routine sizing and counting of coal dust samples at National Coal Board Area Laboratories it is necessary to count particles as small as 0.5 μ. This is at present done with a projection microscope of conventional design with an opaque screen for front projection. In seeking ways to improve this technique we noticed that the picture had to be at least 50 cm from the eye because of physical obstruction of the view by the microscope, so that the magnification used was high, usually about 3000; the picture luminance was therefore very low, about 0.0002 stilb (candle/cm^2), with a 250-w high-pressure mercury lamp as light source.[1] At this low luminance the Fechner fraction is about twice its normal value[2] and the visual acuity is halved,[3] so that it is clearly desirable to increase the luminance considerably.

The obvious way to do this is to use a back-projection screen, so that the distance from the eye to the screen can be considerably reduced, the magnification reduced and the luminance correspondingly increased; but if this is done we find that the grain of the projection screen obscures the detail in the image. All projection screens have a more or less grainy, sparkling appearance, and the scale of the grainy appearance is considerably larger than that of the actual grain in the material. For example, Fig. 1 shows part of a microphotometer trace across a ground glass screen obtained by using a scanning spot 100 μ square and illuminating and collecting beams of N. A. 0.006; the standard deviation of the fluctuations in transmittance is $\pm 23\%$ and it can be seen that the scale of the irregularities is such that detail several hundred microns across would be obscured although the glass was "smoothed" (i.e., ground with the finest grade of emery as the last stage before polishing) and the grain size of the emery was only about 10 μ. The magnitude of the effect also depends on the numerical aperture of the illuminating and collecting beams, the values being chosen here to correspond approximately to conditions in projection microscopy.

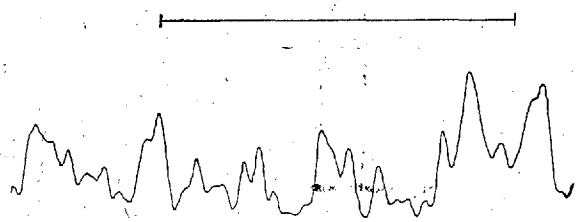

FIG. 1. Microphotometer trace across fine, ground-glass screen. Illuminating and collecting apertures both $f/80$, scanning spot 100μ square. The horizontal line at the top corresponds to 1 mm on the ground-glass screen.

This difficulty of graininess with small numerical aperture of the illuminating beam is found with all kinds of screens to a greater or less extent and it is probably unavoidable. A screen must have irregularities several microns in size if it is to scatter at all and these must be arranged in a random manner so that the screen does not become simply a two-dimensional diffraction grating; it is presumably the linear scale of the random variations in the screen structure which gives rise to the seen graininess, just as the graininess in a photographic emulsion corresponds not to individual grains of silver but to variations in grain density and clumping.

In order to circumvent this difficulty we have therefore applied an old idea[4] for a grainless screen to be used in engineering gauge projectors, etc. In this system two ground-glass screens are placed with their ground surfaces almost in contact and one is moved slowly in its own plane relative to the other; the sparkle and graininess are continually changing and are smoothed out by persistence of vision to give a perfectly grainless, smooth screen. The effect is quite startling for low-contrast objects of which the images are less than a millimeter in size on the screen, such objects are almost invisible when the screens are stationary but become brilliantly clear when the movement is started. The relative speed of the screens needs only to be quite slow, about 1 mm/sec, but the motion must be such that there are no stationary points or else if such a point does occur its effective duration must be less than, say, 1/25 sec; by

[1] This source has a luminance of 20 000 stilbs and the theoretical screen luminance under the conditions of use described above is 0.006 stilb; the difference may reasonably be ascribed to reflection and absorption losses and to the difficulty of completely filling the condenser aperture with the image of the part of the source of maximum luminance.

[2] S. Hecht, J. gen. Physiol. 7, 235 (1924).

[3] S. Hecht, Arch. Opthalmol. 57, 564 (1928).

[4] F. A. MacAdam and Taylor, Taylor & Hobson Limited, British patent 592,815 (1947). See also K. J. Habell and A. Cox, *Engineering Optics* (Pitman Publishing Corporation, New York, 1948), p. 273.

effective duration we mean the time during which the relative speed is less than the minimum for which the grainless effect occurs.

Figure 2 shows an attachment we have constructed for a microscope, using this principle; the front screen moves so that each point on it describes a circle of about 5-mm radius in a few seconds. The gap between the two screens is adjustable and we have found that a separation of up to a quarter of a millimeter has no discernible effect on the quality of the image. An alternative way of moving one screen is to vibrate it at ac-power-line frequency with an amplitude of about 0.5 mm; this is just as effective as the slowly moving rotating screen and perhaps slightly simpler and cheaper.

With this system we have found that an over-all magnification of 1000 permits the finest detail resolvable by means of an oil-immersion objective to be seen as clearly as by direct viewing and the screen luminance is increased to about 0.002 stilb. A further fourfold increase in luminance is obtained if the screens are etched to increase the forward transmission, as proposed by Dyson[5]; the etching produces a clearly visible structure on the screens which can be seen moving, but although this may be slightly distracting to the observer it does not impair the resolution of detail in the image. On account of the strongly peaked polar diagram of these screens it is desirable to use a field lens as indicated in Fig. 2 in order to obtain a uniformly illuminated field of view.

An alternative proposal for a grainless screen is to use a single rather rapidly moving screen[6]; we have tried this in the form of a disk-shaped screen revolving in its own plane and we have found that although an improvement in definition of the image was obtained it was not as marked as for the double screens. The steady movement of the screen through the field of view was always noticed and this was distracting, but worse still was the fact that large-scale variations in scattering over the screen showed up as a flicker with the period of rotation and this could only be eliminated by using a speed of rotation above about 30 rps, a rather high speed for a thin disk of glass. A single, rapidly oscillating screen was found to be quite useless.

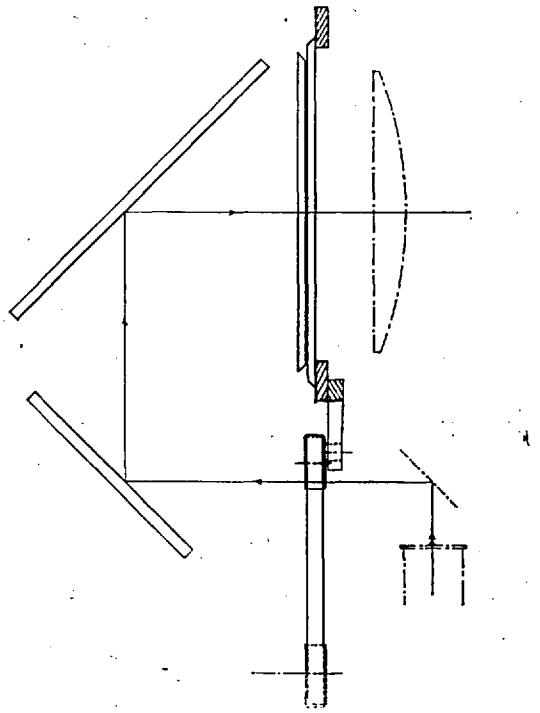

FIG. 2. Grainless screen. Light from the microscope eyepiece (lower right) after reflection from three mirrors forms the image on two ground glass screens (upper center) nearly in contact. The image may be viewed through a field lens (upper right) if the screens are etched for high forward transmittance. The ground-glass screen nearest the observer undergoes circular translation in its own plane at about 20 rpm from the motor drive (bottom center). In an alternative arrangement the moving screen is oscillated in its own plane at ac-power-line frequency.

To summarize, we have found the best results by using two screens separated by not more than 0.25 mm and with relative speed exceeding 1 mm/sec.

ACKNOWLEDGMENT

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[5] J. Dyson, J. Opt. Soc. Am. 50, 519 (1960).

[6] N. H. Mason, British Patent 590,981 (1947). See also E. Lau and J. Reinitz: "Optik aller Wellenlängen" p. 229 (Berlin, 1959) and E. Lau and R. Schalge, Feingerätetechnik 7, 121 (1958).