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A novel method of dark field illumination for a stereomicroscope and its application to a study of the pseudopodia of *Reophax moniliformis* Siddall (Foraminiferida)

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ABSTRACT—A novel system of dark field illumination suitable for stereoscopic microscopes is described in which a beam of light is directed between the objectives. The system is used in an examination of the pattern of pseudopodia produced by the foraminifer *Reophax moniliformis* Siddall in which groups of pseudopodia are often seen to make abrupt obtuse angle changes of direction between about 130° and 160°.

INTRODUCTION

Dark field illumination is a method suitable for studying very fine microscopic detail which is not so readily seen using the conventional Köhler illumination of single-objective microscopes. When stereoscopy is required, dark field illumination becomes more important because the crude system of transmitted illumination which is fitted to some stereomicroscopes is quite unsuitable for viewing satisfactorily such structures as beating cilia or developing pseudopodial arrays. Furthermore, because more refined systems of illumination such as Nomarski differential interference contrast which might replace dark field illumination in single-objective microscopes are not available for stereomicroscopes, dark field illumination itself is essential if such fine structures are to be detected and studied stereoscopically.

The method most frequently used for dark field illumination for stereomicroscopy is one derived from microscopes with a single objective. In this method an optical system which produces a convergent hollow cone of light is placed above the source of transmitted illumination. The specimen being studied is placed at the apex of the convergent cone. The angle and dimensions of the cone are designed so that no light enters the objectives from the hollow cone which diverges from the apex unless an item to be viewed diverts rays into them. Thus, the background is dark and objects are bright, a reversal of the normal pattern using transmitted light, when dark objects are seen on a bright field.

In the present method, a narrow beam of light from a substage source is projected *between* the two objectives of a stereomicroscope so that no light enters either lens when no object is present. Against this dark field, specimens which divert the path of light into the objectives appear very bright.

THE APPARATUS

Design

The apparatus described was designed and built for use with a Kyowa model SDZ-PL stereomicroscope. Fig. 1 shows a diagrammatic isometric projection of the apparatus with the front quadrant removed. The apparatus was built around the lens and iris diaphragm of a camera. One of the components of the compound lens was removed leaving a single lens with focal length 28 mm. The lens and diaphragm were mounted on a set of three discs of Perspex, the lower of which fitted snugly into the depression of the microscope stage in which a glass stage would otherwise have been mounted. The uppermost of the discs, to which the lens and diaphragm are screwed, can be moved relative to the lower discs to allow precise centering of the lens. Above the lens is attached a length of rigid PVC tubing in which are mounted a cone and a disc to interrupt the path of any stray light. A length of slightly larger PVC tubing was made to move smoothly over the inner tube by means of strips of self-adhesive baize. Annular discs of Perspex were machined to fit tightly inside and outside the large tube and together they form the new stage of the apparatus. So that objects on this stage would move freely and without being scratched, the stage was covered with self-adhesive baize.

Fitting into the centre well of the stereomicroscope stage (where a coloured or ground glass filter would otherwise be housed) is an aluminium pot with a hole having the dimensions of the lamp filament centred in its base. The depth of the pot was calculated so that its base would be as close as possible to the lamp. Slight modifications were made to the mounting of the lamp to permit precise centering of the lamp filament.

All potentially reflecting surfaces, other than those of the lens, are covered with a matt, black paint.

The apparatus was designed to be used with the ×2 supplementary objective lens which, with the ×20 eyepiece, gives a range of magnification from 28 to 180 using zoom ratios of 0.7 to 4.5. The working distance is 33 mm.

Use of the apparatus

In any dark field system, stray light reduces the

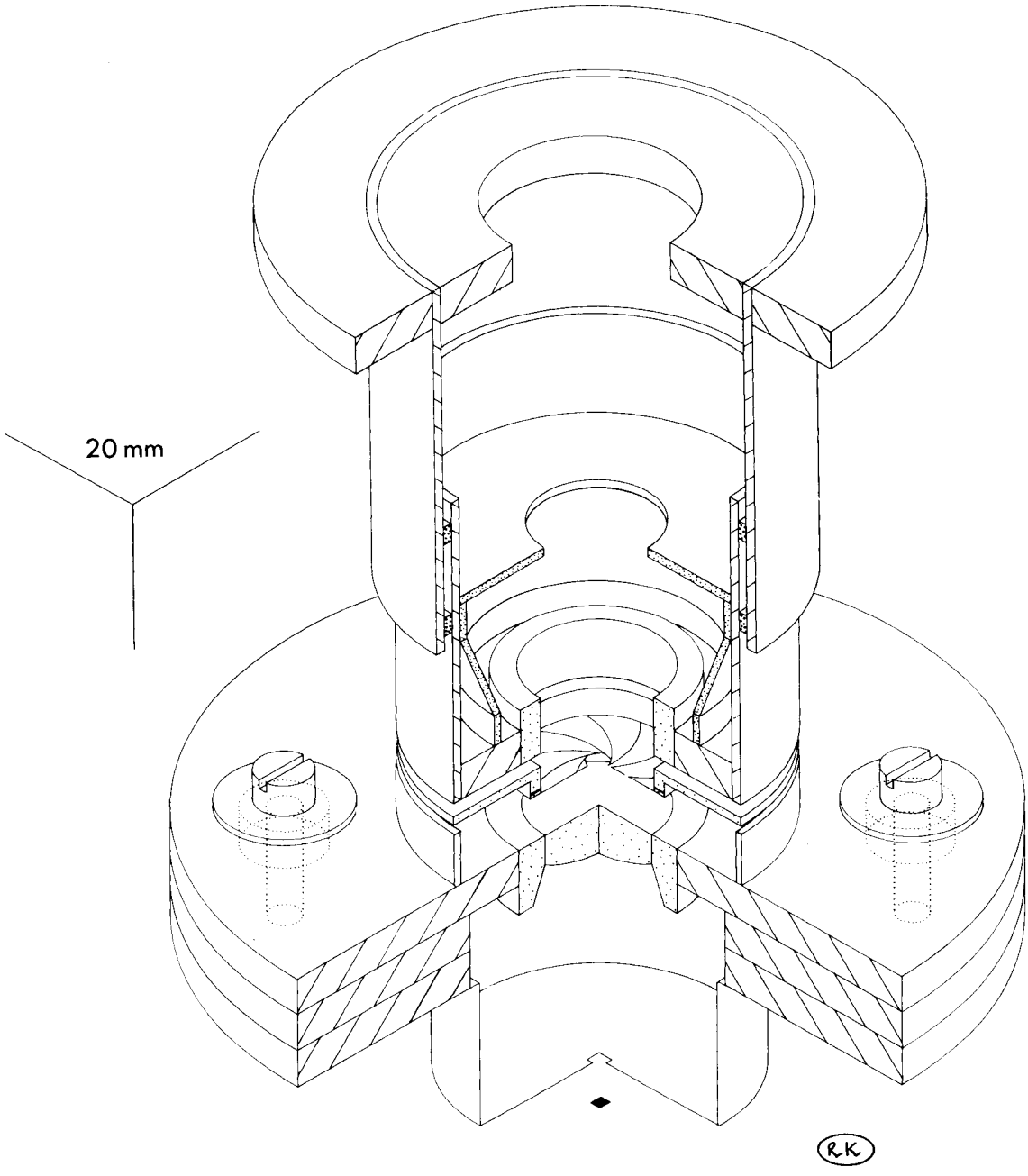

Fig. 1. An isometric projection with the front quadrant removed of the apparatus constructed for dark field illumination. The black square at the bottom represents the lamp filament. Above this is the aluminium can with the hole in the base which restricts the light entering the system. The top of the can is at the level of the stage of the microscope and on this rest the three Perspex annular discs. The uppermost disc can be moved laterally and secured relative to the lower discs by the screw adjustments. The top of the lens is at the level of the top of the uppermost disc; above the lens is the iris diaphragm. The outer of the two PVC tubes moves smoothly over the inner tube so that the image of the filament can be produced either in or out of focus in the focal plane of the microscope. The working stage is formed from the two annular Perspex discs at the top of the diagram. The annular disc two thirds of the way up the inner tube is to restrict stray light as is the cone on which it rests; other structures within and below the inner tube are the mountings of the lens and iris diaphragm which were taken from a camera. The lever for opening and closing the diaphragm is within the cut away part and is not shown.

contrast; the need for scrupulous cleanliness of optical surfaces is therefore paramount. Furthermore, this need extends to the containers for the objects to be viewed and to the medium in which they are to be viewed. All lenses are cleaned before use with distilled water (produced by breathing on them) and lint-free paper. A cautious jet of aerosol propellant effectively removes fine particles. Suitable observation cells for holding living foraminifera have been made from 5 mm thick Perspex machined to give a central hole of 13 mm diameter in a disc of 58 mm diameter. A 26 mm square of double-sided adhesive tape with a hole of 15 mm diameter cut in it is attached centrally to the observation cell and to this is fixed a circular glass coverslip of diameter 22 mm so as to give a watertight seal. The coverslip must be free from scratches and other imperfections. The inner and outer machined edges of the Perspex cell are give a high polish both to facilitate cleaning and to allow lateral illumination of the contained animals when another system of illumination becomes appropriate. The cleanliness of the coverslip is best assured if soft cotton gloves are worn when it is being cleaned and mounted. New surgical gloves which are lubricated with talc are quite unsuitable for this purpose.

Source of foraminifera

Specimens of *Reophax moniliformis* Siddall, 1886 were obtained from a small, brackish pond on a salt marsh in Wacker Lake, a creek off the River Lynher near Antony, Cornwall, UK (Ordnance Survey ref. SX 388 550). The salinity was measured on one occasion as 26‰ but is very variable and greatly dependent on the weather and the point on the tidal cycle. The pond was no more than 100 mm deep and the animals were confined to the top 2 mm of the bottom mud. Their colour indicated that they contained symbiotic algae or their chloroplasts (Knight & Mantoura, 1985).

Animals for viewing were first extracted from the mud by very gentle sieving in sea water which had been collected beyond the breakwater in Plymouth Sound. Individuals were picked out with a pipette or soft-bladed forceps and washed in several changes of seawater. It is essential that no grease from the skin comes into contact with the seawater nor with the containers being used. If, for example, the tip of the pipette being used to transfer the animal from its washing dish to the observation cell is touched with the fingers, there may be transferred to the surface of the liquid in the observation cell a very thin film of grease which will considerably reduce the contrast within the field of view by randomly scattering light.

The shape of the meniscus of the liquid in the observation cell is important as it can effect the direction of the rays of light passing through it so as to divert them into the objectives, and can also give rise to an image of the filament in the field of view. The optimal shape is easily found by gently adding or withdrawing liquid to or from the observation cell respectively with a pipette held almost horizontally.

If the filament of the lamp and the hole in the aluminium pot have both been centred on the optical axis of the microscope, it is necessary only to centre the lens of the apparatus after slackening the two screws on the Perspex disc. This is best done by projecting an image of the lamp filament onto a finely ground glass screen placed on the stage of the apparatus and moving the uppermost Perspex disc until the image is seen to be centred. Final and precise centering is then carried out by opening the diaphragm fully while the zoom ratio is at a minimum and viewing the fields in each eyepiece with nothing on the stage. The fields should appear black. As the zoom ratio is increased, it becomes necessary to close the diaphragm to obstruct light which would otherwise enter the objectives. The final adjustment of the position of the lens is made during this process so that the left and right fields appear identical.

Dark field illumination and stereoscopy

When single-objective microscopes are used with dark field illumination, a condition of symmetry exists between the light source, the specimen and the objective. No matter what the alignment of the specimen relative to the lens, light reflected from or refracted through the specimen at certain angles will enter the objective. However, in stereoscopy this symmetry is missing as the objective lenses are set to one side of the axis of the light path. In the present apparatus, in which the angle between the incident and the diverted light is small, the orientation of a straight line specimen has a marked effect on its appearance under dark field illumination and this difference is increased because the brightest images are formed from the edges of specimens. When the long axis of the specimen is at right angles to a line joining the centres of the two lenses, a maximum amount of light enters the lenses from the edges of the specimen; however, when the long axis of the specimen has been turned through 90° most of the light diverted from the edges does not enter the objectives at all. The resolution of the specimen in the first position is poor because of the brilliance of the image; by the same token, the fineness of the specimen which can be detected is maximal. In the second position, although uniformly thin pseudopodia are not so readily detected, the resolution of discontinuities such as the nodules which stream up and down the pseudopodia is greatest. It is important, therefore, that during stereoscopy using dark field illumination, the lens systems or the observation cell should be turned through 90° from time to time. Because of the fragile nature of a pseudopodial array and the sensitivity of foraminifera to abrupt movements, the former is less likely to alter the system under surveillance.

The present apparatus is thus complementary to the

type in which a hollow cone of light is used. In the latter apparatus, in which the angles between the incident rays and the diverted rays which enter the objectives are greater, the orientation of specimens relative to the lenses of the microscope is of less significance; as a consequence the components of a field are more evenly illuminated. The advantage of the present system lies in the fact that a choice may be made between the degree of resolution and the brilliance of any part of the field.

PSEUDOPODIAL PATTERNS IN *REOPHAX MONILIFORMIS* SIDDALL, 1886

Accounts, from Dujardin (1835) onwards, of the pseudopodia of foraminifera are not lacking. Hedley (1964) included in his commentary the words, "Following a century devoted to marvelling at the wonders and beauty of the foraminiferal reticulum . . .", and the present author admits to having had his share of wonderment. An extensive review of the pseudopodia of foraminifera was given by Sheehan & Banner (1972). However, the pseudopodia of *R. moniliformis* appear not to have been studied hitherto and no mention is made in the literature of the occurrence in other species of the reflexed structures described below (Prof. F. T. Banner, pers. comm.). It should never be forgotten, however, that the conditions under which foraminiferal pseudopodia are best observed may bear no relation to the natural environment of the animals and that pseudopodial arrays which they make in response to the entirely artificial environment of the laboratory may not necessarily be made in nature.

When a specimen of *R. moniliformis* is placed in an observation cell, it aligns its test normal to the surface with which it is in contact by means of the developing pseudopodial array, which issues from the aperture (Pl. 1, figs. 1, 2). Animals will generally align their long axis parallel to the axis of the microscope and then move horizontally; however, if the animal's aperture is close to a vertical surface, say, the sides of the observation cell, it may well attach to this surface and climb the wall of the cell while holding the test horizontally.

Having arranged itself normal to the surface, the foraminifer collects from its surroundings particulate material which it moves along the pseudopodia to be stored around the aperture. At the same time, it frequently moves sideways. Although millipore-filtered seawater is used as the medium in which the foraminifer is observed, some particulate matter is inevitably transferred to the cell because it is not possible to clean completely a specimen of this agglutinating foraminifer without undue risk of damage to it. Moreover, this particulate matter is augmented, especially during long periods of observation, by particles settling from the air. Pl. 1, fig. 1 shows a freshly cleaned specimen which has

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Explanation of Plate 1

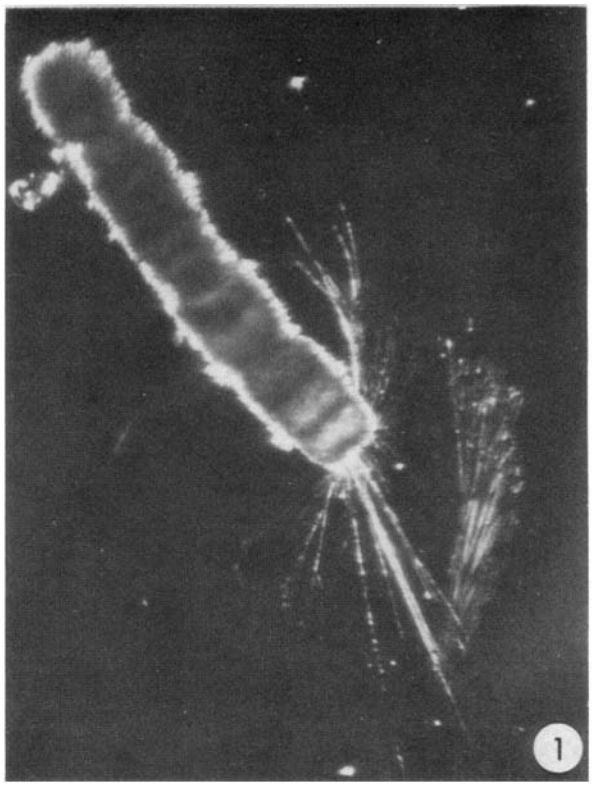
Pseudopodia of *Reophax moniliformis* Siddall photographed under dark field illumination, as described herein. Scale bars are not included on the plates nor were measurements made of the specific animals used in this study. However, the diameter of the chambers of *R. moniliformis* from the same source as those figured here lie between 100μm and 150μm and these values may be used to give an approximate scale.

Fig. 1. Specimen 1. This animal had arranged itself vertical to the floor of the observation cell and had produced an array showing an obtuse angle change of direction before it toppled over to lie momentarily horizontally, when the photograph was taken. This and other figures illustrate the fact than an array of reflexed pseudopodia does not necessarily originate in a single node but frequently arises from several pseudopodia which undergo reflexion close to each other. The acute angle changes in direction of the pseudopodia of the reflexed array which are closest to the test are a common feature of the arrays produced by several species of foraminifera and may be brought about by the adherence of pseudopodia to the substrate at a certain point followed by movement of the animal together with the proximal parts of the pseudopodia which have not adhered.

Fig. 2. Specimen 2. This specimen is vertical and gives rise to a single reflexed array from a single point on the originating pseudopodium (bearing 145°). The reflexed array is crossed and not disturbed by the bright pseudopodium which is roughly parallel to the originating pseudopodium.

Fig. 3. Specimen 3. The pseudopodia bearing about 190° are reflexed to give rise to part of the array on the left of the picture. Other parts of this reflexed array appear to arise from pseudopodia bearing about 203° and 220° respectively. Notice the other pseudopodium bearing 220° which branches distally and which crosses, without apparently interfering with, the reflexed arrays.

Fig. 4. Specimen 3. A photograph of the same specimen taken a short time later. This picture was taken in a different orientation after the animal had moved slightly; the original array has been completely replaced. Two reflexed arrays bearing 30° and 15° respectively originate from pseudopodia bearing 160°. Notice that the originating pseudopodia of the latter continues its course after giving rise to the reflexed limbs.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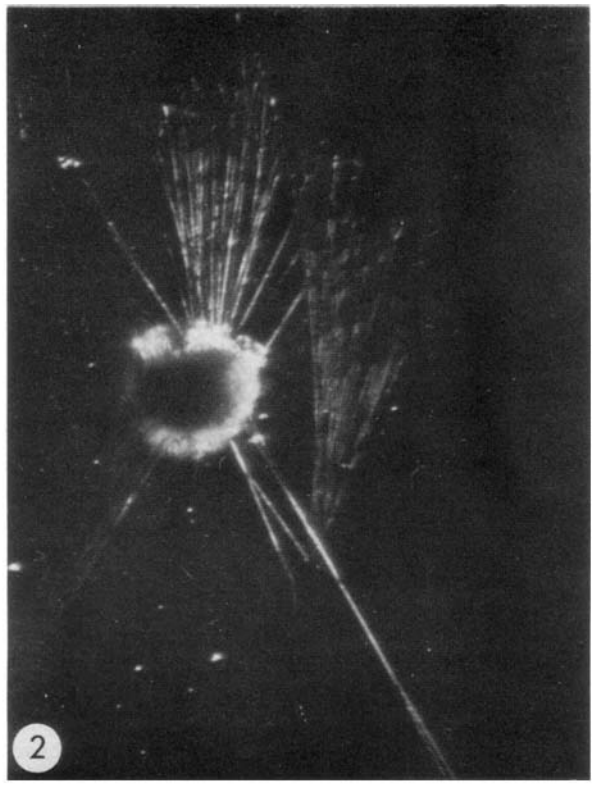
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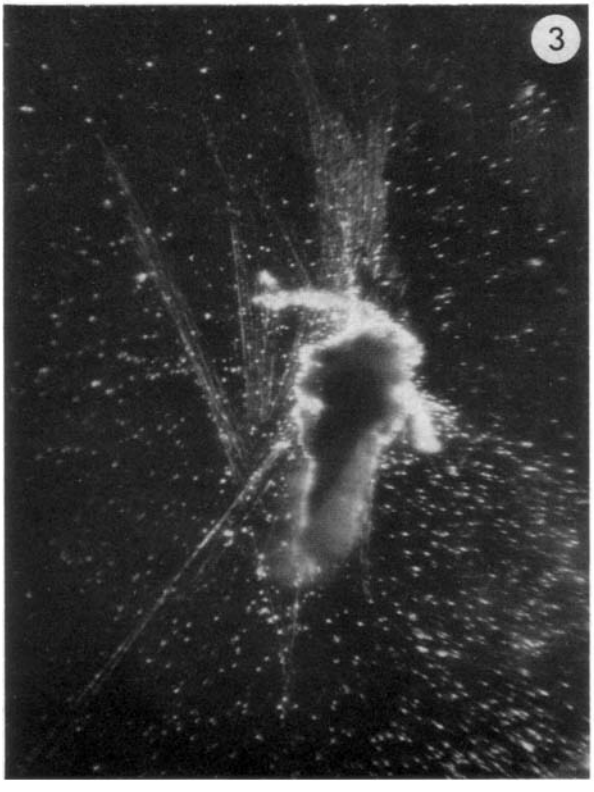
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not collected particulate material around it whereas in all the other figures collected debris is in evidence.

The pseudopodial array is ever changing and features of it, such as obtuse angle changes in direction, are no less evanescent than other aspects of the array (Pl. 1, figs. 3, 4). A full description of the generation and the subsequent degeneration of an array is outside the scope of the present report and the figures merely capture moments during the cycle when obtuse angle changes in direction of pseudopodia are evident. It is important, however, to realise the limitations of still photographs. Apparent junctions between pseudopodia travelling in different directions may be spurious and the apparent lack of a visible junction may not always reflect reality but may be an effect of the dark field system of illumination when shadows cast by one object may have a significant effect on the appearance of another.

Acute angle changes in direction of pseudopodia can be brought about by movements of the animal (Pl. 2, figs. 1, 2,3). Such an acute angle bend is seen in Pl. 2, fig. 1 in the pseudopodia bearing about 165° and this angle increases progressively from about 10° to about 25° in the series as the foraminifer moves to the left. Clearly, the pseudopodia are attached to the surface at the bend and as the animal moves, it carries with it the free, proximal part of the pseudopodia. Unless the foraminifer moves directly towards or away from the point of attachment of the pseudopodia to the substrate, it will create an angle between the parts of the pseudopodia fixed to the substrate and the parts carried with it. The maximum angle which we have observed to be formed in this way is about 25°.

Smooth curves in single pseudopodia or in an array may occur in some species if the observation cell is jolted, and this effect is seen in part of the array of Pl. 2, fig. 3.

Obtuse angle changes in direction often appear to occur from several points in close proximity on an originating pseudopodium or may occur in close proximity to each other from a bundle of separate pseudopodia. In one case (Pl. 1, fig. 4) at least one of the originating pseudopodia bearing about 160° continues its course after giving rise to the reflexed limbs.

It is noteworthy that abrupt changes in direction of pseudopodia between about 25° and 130° have not been seen. The mechanism for the obtuse angle changes appears not to produce a wide range of angles; it might be expected, however, that the movement of the animal which produces the acute angle changes in direction could produce much greater changes in angle than have been observed.

The function of the obtuse angle changes of direction in parts of the pseudopodial array of *Reophax moniliformis* is obscure. No association between their occurrence and any external factor has been detected. All that can be said is that this facility allows *R. moniliformis* to feel around corners. The possibility that they are only produced in response to a laboratory environment must not be overlooked and it would certainly tax an observer bent on determining whether or not they are produced in this foraminifer's natural environment, in the mud at the bottom of a brackish pond.

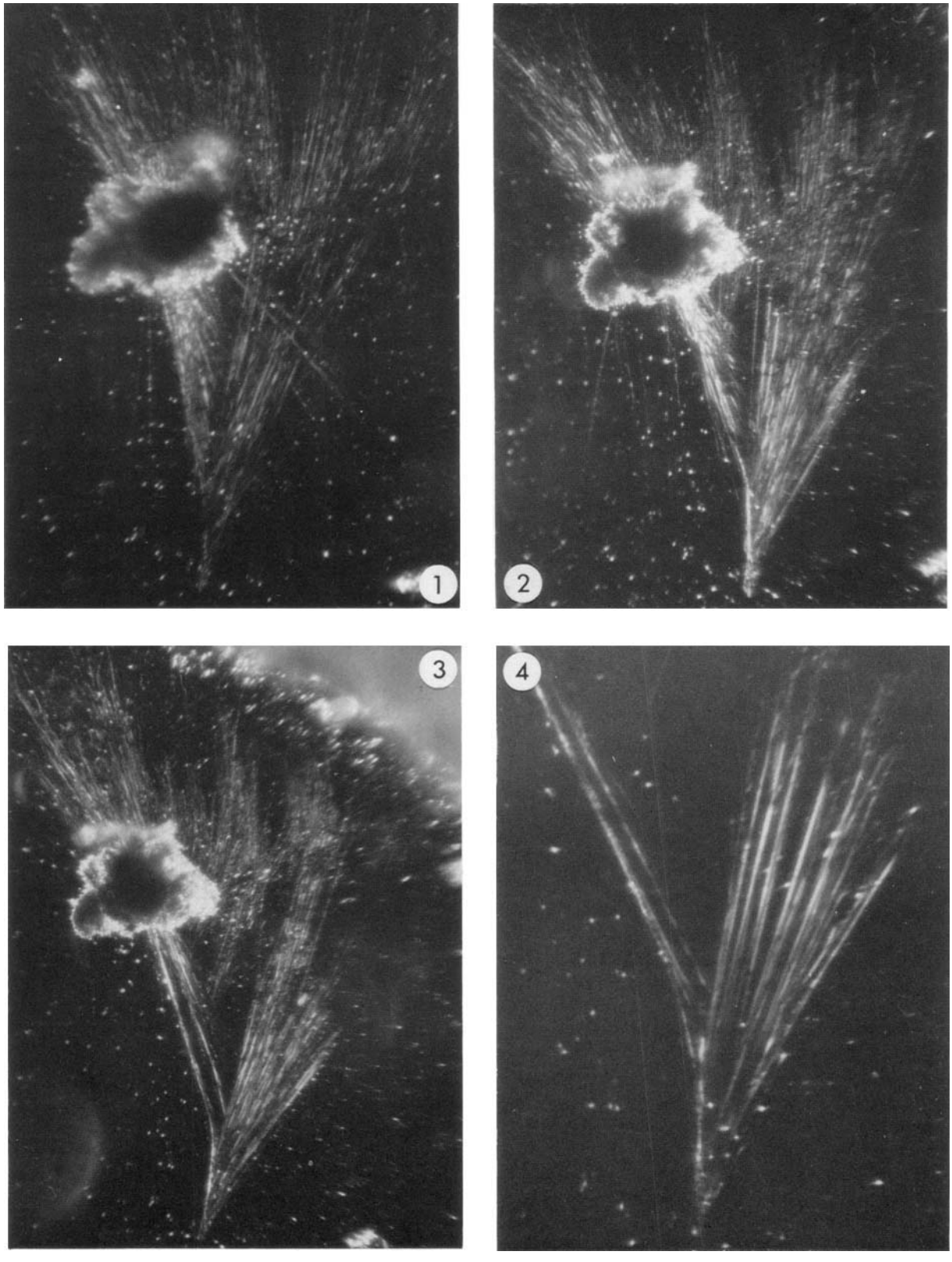
It may be noted that this member of the Textulariina produces a pseudopodial array which emanates solely from the aperture and thus differs markedly from certain members of the Rotaliina in which pseudopodia emerge from the aperture, the umbilicus, the retral fossettes and the perforations of the wall (Sheehan & Banner, 1972; Banner & Williams, 1973). (The only occasions when pseudopodial arrays have been seen to issue from a part of the test other than the aperture in *R. moniliformis*

Explanation of Plate 2

Pseudopodia of *Reophax moniliformis* Siddall photographed under dark field illumination, continued. Scale bars are not included on the plates nor were measurements made of the specific animals used in this study. However the diameter of the chambers of *R. moniliformis* from the same source as those figured here lie between 100μm and 150μm and these values may be used to give an approximate scale.

Figs. 1, 2, 3. Specimen 4. Successive stages in the development and regression of an array. The animal holds itself vertically and the aperture, which is applied to the substrate, is surrounded by collected material. The reflexed array in fig. 1 is crossed but not disturbed by several pseudopodia bearing about 140°. An acute bend in the pseudopodia bearing 165° is seen close to the most proximal of the reflexed pseudopodia and this acute angle increases progressively from 10° to 25° in the series as the foraminifer moves to the left. At the same time the most distal of the reflexed pseudopodia are foreshortened and the bundle of pseudopodia from which the reflexed limbs arise are resolved into two major and several smaller pseudopodia. In figs. 2 and 3 it can be seen that the array in the centre top of the pictures and bearing 0° to 355° consists of reflexed branches of pseudopodia bearing 160° and emanating from the test.

Fig. 4. Specimen 4. A detail of a later stage than in fig. 3 showing how the reflexed pseudopodia start from several points along the course of the originating pseudopodia.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have been when animals have been damaged during manipulation, when pseudopodia have been seen to originate at the points of damage). The fundamental differences in the structure of the tests of the two suborders make it possible that this will be found to be a constant difference between them, but no definite conclusions can be drawn without an examination of further textulariid species, both perforate and imperforate.

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