SVERIGES GEOLOGISKA UNDERSÖKNING

SER C NR 641 AVHANDLINGAR OCH UPPSATSER ÅRSBOK 63 NR 4

K. E. SAMUELSSON

ELEMENTS IN MICROPHOTOGRAPHY

WITH ORDINARY LIGHT

STOCKHOLM 1969

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C. DAVIDSONS BOKTRYCKERI AB, VÄXJÖ

CONTENTS

Abstract. This illustrated guide and reference manual contains instructive advice and hints which the author has found in his own experience to be of value in microphotography, irrespective of the scientific field and equipment used. The points dealt with include the more important, such as optics, light and diaphragm, as well as those of slightly less importance like detail analysis, negative material and exposure time – but all of which not only contribute in producing a good result but also greatly facilitate the work of microphotography.

PREFACE

During the last few years I have had many inquiries about microphotography, and hence this work is an attempt to meet the need for a manual on the subject. Short and casual visits, correspondence and telephone conversations on this theme are quite inadequate. The numerous factors involved together contribute to true and succesful documentation. The recommendations and information given in this work are based on the author's own experiences in microscopy and photography. It is hoped that this short guide will prove useful for beginners, while at the same time act as a reference book for the consultation of the somewhat more experienced.

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INTRODUCTION

Hesitation and lack of knowledge about the basic elements contributing to a true and successful photograph can make it difficult to obtain the desired result. However, with sufficient information the microphotographer has abundant possibilities to reproduce the details of complicated objects, depending on what a light microscope is able to separate. Lighting, diaphragm and optics produce a true picture; negative materials colour filters, embedding agents and correct time of exposure all contribute in creating a successful photograph.

The equipment generally used is a fixed microscope stand with binocular supplement and a tube-extension for the camera. Optics and – if desirable – phase contrast equipment from a well-known manufacturer. Modern equipment includes low-tension and Xenon lighting, a camera with plate holder for films or plates, size 9×12. Other parts of the equipment are exposure meters, filter assortment, object micrometers (callipers) and immersion oil. Tables of exposure, choice of negative materials and colour filters should also be included.

ANALYSIS OF THE DEGREE OF DETAIL

The microscopic objects to be photographed may be divided into two groups, partly complicated, partly uncomplicated. With the complicated objects detail can be shown in two or more layers, while the uncomplicated have them in one layer only. All objects have in common the fact that one detail as a rule can be brought into focus in two positions – one high and one low. With an embedding medium having a lower light refraction index (n B) than the object, portuberances or spinules turn out light at high focus and dark at low. In case of depressions or cavities the result is exactly the reverse, at high focus dark and at low focus light. Should the object be complicated, the same result is obtained for each layer as for the exterior details. The details separating the layers turn out light at high focus and dark at low. In the case of cavities the result is the reverse, dark at high and light at low focus. Apparently breaking this rule are indentations or cavities on the underneath side of the object. They also become light at high and dark at low focus. This can probably be explained by the fact that the embedding medium acts as an object with protuberances and the object consequently becomes the embedding medium.

Should, however, the embedding medium have a higher refraction index than the object, the whole pattern becomes reversed, protuberances or spinules turn out dark with high focus and low with light etc.

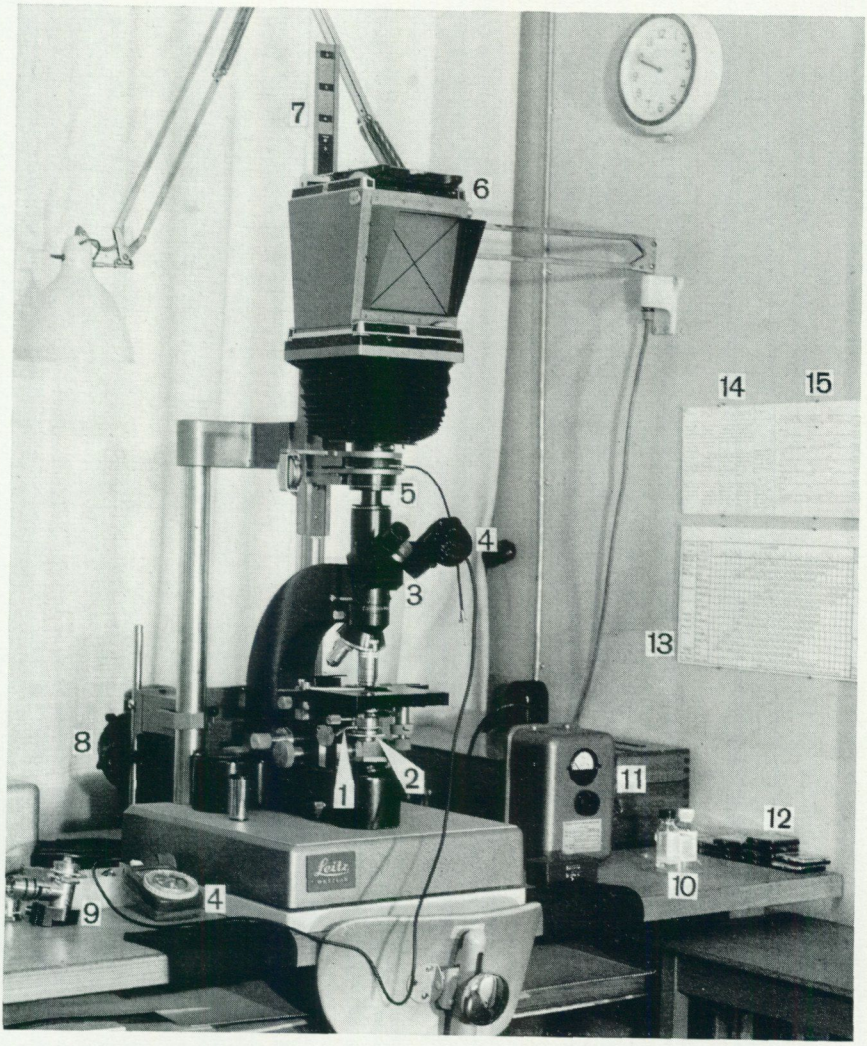


Fig. 1. 1. Diaphragm. 2. Light-field diaphragm. 3. Visual eyepiece. 4. Leitz exposure meter Microsix L. 5. Camera eyepiece. 6. Plateholder. 7. Scale markings on stand for elevation of folding camera. 8. Xenon lighting. 9. Opaque illuminator. 10. Immersion oil. 11. Transformer for low-tension lighting (lamp obscured). 12. Filters. 13. Table of exposure. 14. Plate selection table. 15. Filter selection table.

With this possibility of choosing high or low focus and with the support of suitable intensity of light and a correctly adjusted aperture, the microphotographer is well able to substantiate details of rather complicated objects.

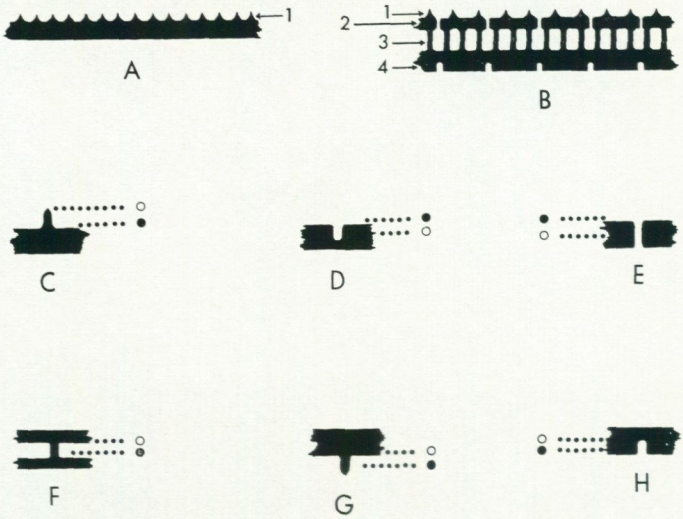


Fig. 2. Schematical drawings of section. A. Simple object with details in one layer (1). B. Complicated object with details in four layers (1–4). C. Ridges, spinules or similar projections are light at high focus and dark at low. D and E. Depression and hole become dark at high focus and light at low. F. Details separating two layers become light at high focus and dark at low. G. Protuberances on the underneath side of the object become light at high focus and dark at low. H. Cavities on the under side of the object are light at high focus and dark at low.

Remark: Should the embedding medium have a higher refraction index than the object, the pattern becomes the reverse.

..... = Approximate focus level.
o = Detail lighter. • = Detail darker.

EMBEDDING MEDIUM

As a rule the microscopic objects are refractive and usually display colour as well as contrast. In some cases there are only refraction-contrasts and, if they are very small and weak – even when using a very hard-working plate – difficulties in reaching a satisfactory documentation may occur. This can be improved by using a suitable embedding medium. The contrasts improve, if the refraction index of the embedding medium (n B) to a great extent diverges from that of the object.

In photographing diatoms an embedding medium with a high index (n B) = 1.71 and 2, has been used for very fine details and a low index (n B) = 1.33 for recent pollen and spores with faint details and a very faint colour. With regards to these objects the results have been satisfactory, but when considering other

tasks, it has been evident that objects with dark, dense colours turn out darker still, if the refraction index of the object and embedding medium differ too much. Difficulties in obtaining desired details using filters may also occur.

Examples of embedding media and their refractive indices:

Water	n B = 1.33
Silicone	n B = 1.38–1.53
Glycerine	n B = 1.42
Glycerine-gelatine	n B = 1.47
Canada-balsam	n B = 1.50
Hyrax	n B = 1.63–1.65
Clearax	n B = 1.66
Lurifax	n B = 2.00

OPTICS

In microphotography one may on the whole choose between a general or detailed picture. The general picture is mostly taken with opticals of low rate of enlargement and low numeric aperture and – in order to get a depth of field in certain cases – an aperture, not always suitable for the details of a complicated object, is used. A general picture of a complicated object should not be enlarged and accepted as a detailed picture – the details may be somewhat mixed.

For the detailed photograph an objective with a high resolution lens is needed. The numerical aperture for the extreme enlargements must be 1.32 or 1.40. Optics with an aperture of 1.40 have as a rule a short working distance and can only be used for slides with thin coverglasses. For older slides with thick cover-glasses, optics with longer working distance must be used and consequently a somewhat smaller aperture = 1.32 is obtained.

The resolution of an objective is decided by the formula $d = \frac{\text{wavelength}}{\text{n. ap.}}\ \mu$. With transmitted lighting centred in the optical axis the smallest reproducible detail is:

Wavelength	n. ap.	Smallest resolvable detail
0.400 μ	1.40	0.285 μ
0.700 μ	1.40	0.500 μ

If the focus is removed out of the optical axis the resolving capacity can be increased with component $2\,d = \frac{\text{wavelength}}{\text{n. ap. } 2}\ \mu$.

Wavelength	n. ap.	Smallest resolvable detail
0.400 μ	1.40	0.143 μ
0.700 μ	1.40	0.250 μ

The same formula applies to the distance between the details.

In publishing a general picture, which has been enlarged, it would be advisable to indicate the method used. For a picture taken in X400 and enlarged X3, the resulting enlargement is $400 \cdot 3 = X1200$.

PRINCIPLE: When microphotographing the details of a complicated object use optics with the greatest possible numerical aperture.

LIGHTING

It is advisable to use low tension lighting equipped with an adjustable transformer, as a general light source. With for instance 6 V, 6 amp. a colour temperature of 2800 (°K) is reached, with 5.4 amp. 3000 (°K) and with 5.7 amp. 3200 (°K). If the strength is increased to the limits of the capacity of the lamp (6.1 amp.) the result is 3400 (°K). The lowtension lighting is poor in violet and blue. For some objects it may be of advantage to use a lighting with higher radiation of violet and blue. The Xenon lighting's (Xenon-Brenner XBO 150) colour temperature is about 6300 (°K) and its spectral curve is even within the entire visible light.

The strength of lighting is, especially regarding enlargements, an important factor. In order to obtain an exact, true photographic picture of the details in a complicated object, a high intensity of light must be used. A strong light should, together with a suitable aperture (see the diaphragm) ensure that the details below or above are eliminated, so that only the desired details in the absolute objective field are reproduced. (The "objective field" is the picture of an object visible in the microscope, when the diaphragm has its widest possible active opening.)

For a general picture the strength of illumination is not of the same importance, but in most cases a somewhat weaker light and a smaller aperture is appropriate.

The lighting point in the illumination must as a rule be centred in such a way that it coincides with the optical axis. When the lamps are changed, centering must also take place. For simpler details (striated or dotted) the resolution ability in the objective can be increased by a factor of 2, if the lighting point in the illumination is removed from the optical axis, so that the light falls aslant into the condenser.

PRINCIPLE: In detail photography of complicated objects the intensity of the illumination should be high.

THE DIAPHRAGM AND THE LIGHT-FIELD LIMIT

In amateur-, advertising- and portrait-photography the diaphragm is used as an aid in reaching a certain depth of field, but a photographer having more or

less transparent objects must, on the other hand, use the diaphragm with great care. If the specimens are complicated with details in different layers, these might be muddled and result in an incorrect picture, if the diaphragm is too "hard" (small aperture). One and the same object can, in different photographs with quite different apertures (small or carefully adapted), give such results that they cannot be identified as the same object. With the use of a diminished aperture other disagreable consequences follow, i. e. dark shadows around the details, preventing the estimation of their actual size, and with an extremely diminished aperture fine details can be overlooked in microscopy as well as in microphotography.

An optical illusion might also ensue, especially in photography of round objects, such as pollen, spores or similar material. The picture appears inside the object and turns out more contrasted the smaller the aperture. The details of these objects consist of very small lenses and if they for instance are biconcave, the result is a reduced picture of the details of the object itself. If the upper part of these objects is removed, there is no optical illusion.

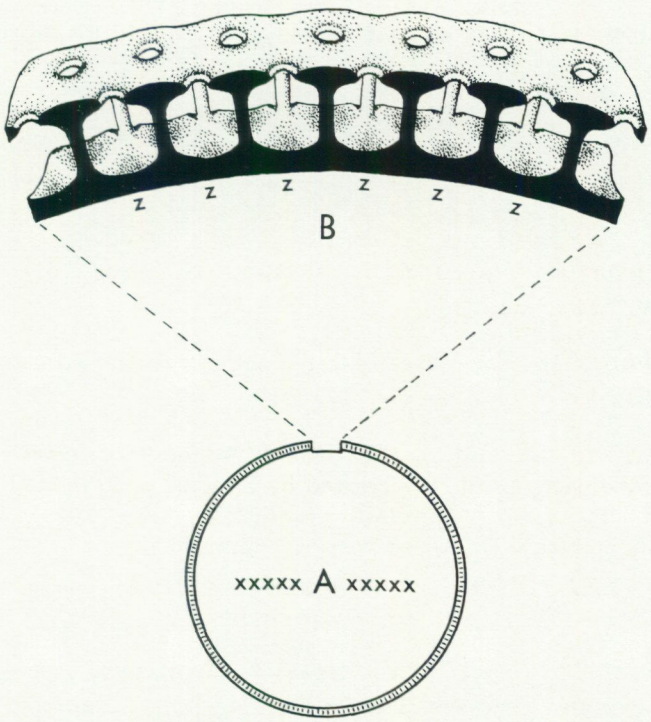


Fig. 3. Schematical drawings of round object, which might produce an optical illusion. A. Optical section of the object. B. Enlarged section (the visible surface quite black).
xxxxx = Approximate place for the optical illusion.
z = Small biconcave lenses.

It is impossible to give any general advice about the aperture, the correct choice of aperture being a matter of experience. For the intricacy of detail of complicated objects, plate and filter play the greater part in producing the desired contrasts – not the diaphragm. The light-field condenser is focused so that it becomes similar to the resp. objectives aperture (opening).

PRINCIPLE: Utilize – as far as possible – the whole resolution ability, adjusting the diaphragm with great care.

THE NEGATIVE MATERIAL

It might be difficult to obtain a desired result, if one hesitates in the choice of negative. Knowledge of the types of emulsion available on the market facilitates the task to a great extent. The attributes to be observed primarily are the colour sensitivity of the material, its gradation and granularity.

The colour sensitive negative material is divided into four emulsion types:

Non colour sensitive material. Sensitivity in violet and blue, decreasing in bluish green and discontinuing in green.

Orthochromatic material. Sensitivity in violet, blue, green and yellow, which soon discontinues in orange.

Panchromatic material. Sensitivity in the entire visible spectrum, violet, blue, green, yellow, orange and red.

Infra-red sensitive material. Violet, blue, red and ultra-red, (frequently a sensitivity space between red and blue).

With regard to gradation we distinguish between:	Division into granularity gives us:
hard working material	very fine grain
normally working material	fine grain
soft working material	normal grain

As appears from the above all four emulsions are sensitive to violet and blue. The same possibility does not exist for the remaining colours. For green and yellow only two emulsions, the orthochromatic and the panchromatic. For orange and red only one, the panchromatic, and for ultra-red only the infra-red emulsion remains.

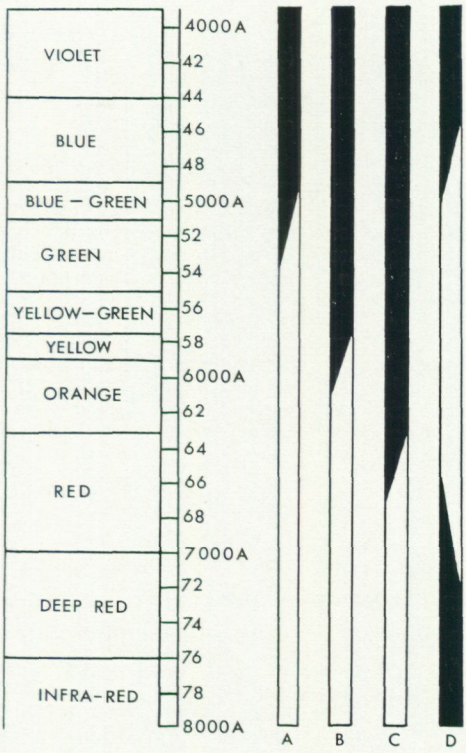


Fig. 4. The approximate areas of sensitivity (black) of the four emulsion types. A. Non-colour-sensetive plate. B. Orthochromatic plate. C. Panchromatic plate. D. Infra-red sensetive plate.

In order to facilitate the choice of emulsion for the inexperienced it is simplest to start from the colours of the object. If the object is coloured blue, green and red, a panchromatic emulsion must be used. In the case where the only colour is yellow, the orthochromatic emulsion with its more pronounced sensitivity in yellow may be selected and for violet and blue the non colour sensitive emulsion.

At times it may be necessary to choose the emulsion according to the colour of the filter. Should the object for instance show a very weak violet-blue colour in the details, a filter of a complementary colour (yellow) must be used in order to get a contrasted picture.

GUIDE TO THE CHOICE OF SUITABLE NEGATIVE MATERIAL

Details	The colour or colours of the object			
	None, violet, blue	Violet, blue, green, yellow	Orange, red, etc.	Ultra red
Very small and faint refraction contrasts. On the borderline of the resolution ability of optics	Hardworking (slow) non colour sensitive plate with very fine grain nr. 1	Hardworking (slow) orthochromatic plate with very fine grain nr. 4	Hardworking (slow) panchromatic plate with very fine grain nr. 7	Hardworking (slow) infra-red plate w. very fine grain nr. 10
Comparatively sharp a. easily discernible refraction contrasts	Normally working non colour sensitive plate w. fine grain nr. 2	Normally working orthochromatic plate w. fine grain nr. 5	Normally working panchromatic plate w. fine grain nr. 8	
Sharp and dark refraction contrasts	Softworking, non colour sensitive plate with normal grain nr. 3	Softworking orthochromatic plate with normal grain nr. 6	Softworking panchromatic plate with normal grain nr. 9	Softworking (fast) infra-red sensitive plate w. somewhat larger grain nr. 11

The idea, however, is not to use all the above illustrated plates. The guide is intended as a help in choosing the most suitable plates for the objects involved. Very variable material may necessitate the use of five to six of these.

EXAMPLES OF NEGATIVE MATERIAL

No.	Film	Wavelength area mμ	Speed	Contrast	Grain
1	Thin film half-tone	230–530	slow	very high	very fine
2	Ordinary plate	230–520	normal	normal to high	fine
3	Soft ordinary plate	230–520	normal	normal	fine
4	Ortho half-tone plate	230–580	slow	very high	very fine
5	Chromatic plate	230–580	normal	normal to high	fine
6	Selochrome plate	230–580	fast	normal	normal
7	Thin film halftone Panchromatic plate	230–660	slow	very high	very fine
8	Special Panchromatic plate	230–650	normal	normal	fine
9	Soft gradation Panchromatic plate	230–650	fast	normal	normal
10	Infra-red process plate	740–880	slow	high	very fine
11	Infra-red Extra Rapid	670–880	fast	normal	fine

1–10 = Ilford; 11 = Kodak.

PRINCIPLE: The colour or colours of the object decide the type of emulsion, the details determine, whether a hard-, normal- or softworking plate is to be used.

FILTER

In microphotography colour filters are used to a very great extent. Frequently occurring filters are pale blue, blue or bluish green, pale green, yellowish green, pale yellow, darker yellow, orange, light red, dark red and infra-red.

There is a prevalent profusion in the working symbols of a filter. A filter, which within one field of research is called contrast filter, can within another be known as colour compensating filter, although both lead to the same result: sharper contrast in the picture.

A more standardized terminology is needed and the filters should, in accordance with range of application and the influence of the filter effect on the negative material, be divided into four groups:

Focusing filters
Object colour filters
Colour compensating filters
Correction filters

Blue and blue-green count as *focusing filters* and are used in focusing in that wave range, for which "the non colour sensitive emulsion" is susceptible. The yellowish-green filter, which must be used for achromatic objectives, also belong here.

Filter of the same colour as the object or its details should be called object colour filter and are the cause of the object and its details coming out lighter with than without filter.

For a darker picture a colour compensating filter should be used.

The lighter filters are as a rule put in as correction filters and used in distinguishing between two colours, which in photography have turned out in the same grey colour range.

The colour of the object or details	Object colour filter	Colour compensating filter	Results
Violet	Violet –	– Yellow	Lighter Darker
Blue	Blue –	– Orange	Lighter Darker
Green	Green –	– Red	Lighter Darker
Yellow	Yellow –	– Violet	Lighter Darker
Orange	Orange –	– Blue	Lighter Darker
Red	Red –	– Green	Lighter Darker
Infra-red	Infra-red	–	Lighter

Examples of filters:

Pale blue (usually provided with the microscope)			
Blue-green	Kodak	Wratten	No. 75
Pale green	„	„	No. 56
Yellowish green	„	„	No. 11
Pale yellow	„	„	No. 6
Dark yellow	„	„	No. 16
Yellowish orange	„	„	No. 22
Light red	„	„	No. 23A
Dark red	„	„	No. 29
Infra-red	„	„	No. 89B

PRINCIPLE: In order to the colour absorbing qualities of the emulsion types, filters should always be used in microphotography at high magnifications.

TIME OF EXPOSURE

A correctly selected time of exposure is just as important as the other factors. Under- or strong overexposure always mean failure. With the help of an exposure meter, used with discernment, the earlier problems with exposure can be entirely avoided. For those, who have variable specimens and work with all emulsion types and filters, it is advisable to work out a table of exposure.

In order to fix the time of exposure at different bellow extensions, the marker must be graded. To do so the camera (with bellows) is shut up as far as possible. Thereafter the marker is divided with a line every four centimeters, beginning on a level with the plateholder – the divisions being marked from below with 0, 1, 2, 3, 4, etc.

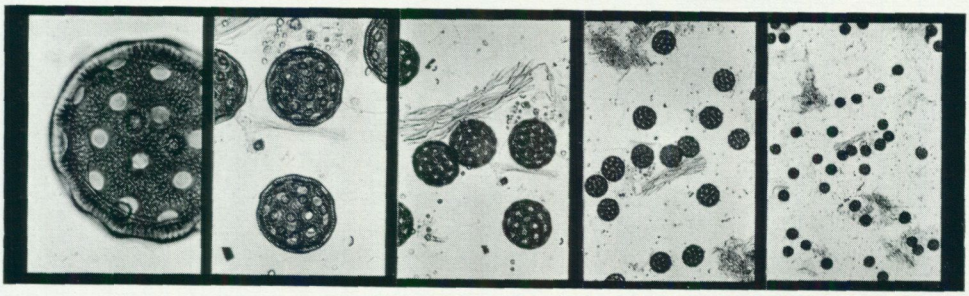
For the purpose of avoiding several exposure times because of the different sizes of the objects (at the same degree of magnification) it is appropriate with test-photography to use an object filling half the plate. In that way it is possible to use the same time of exposure for small as well as large objects – the latitude of exposure in black-white being quite sufficient in such cases.

With the camera in O-position the test-photography can proceed. If the light-value in the visual eyepiece is 10 (measured with Leitz Micro-six L) and the time of exposure – in case a panchromatic plate and a pale blue filter are used – is found to be 16 seconds in order to obtain a well exposed negative, all necessary times of exposure for all light-values are therewith evident, i.e. for above mentioned plate and filter.

Example:

Plate	Filter	Lightvalue								
		etc.	11	·	·	10	·	·	9	etc.
Panchromatic	Pale blue	etc.	8	10	12	16	20	24	32	etc.

Should the camera bellows extension be pulled out for instance to 2 on the marker – the time of exposure must be extended two places to the right. If the light-value is ten and the above mentioned plate and filter are used, the time is 16 seconds; with two places to the right the correct time of exposure for the extension = 24 seconds. For each mark that the camera is raised, one place to the right on the table of exposure should be taken.



100X. 40X. 25X. 12,5X. 6X.

Fig. 5. Change of objective does not give any alteration of the times of exposure in the tabular statement.

Only a change in the visual eyepiece leads to an alternation in the time of exposure. If eyepiece X6 has been used in the calculation of the table and a change is made for instance at X10, there must be a time prolongation. If the time for eyepiece X6 equals 16 seconds, the time (x) for eyepiece X10; $\frac{6}{16} = \frac{10}{x} = 27$ seconds.

DEVELOPERS

To find a common developer for all emulsions should not be difficult. Every manufacturer has one of this kind for the "non colour sensitive", the orthocromatic and the panchromatic plates. The developer should also – with an eventual change in the time of exposure – be used for the infra-red sensitive plate.

The enclosed tables of exposure are a recommendation and should on the whole be followed.

For single "shots" a developing dish may be suitable, but those who work more continously should use a tank for developing.

PRINCIPLE: Always develop at the same temperature.

The most commonly used plate for fossil as well as recent *pollen and spores* are the normally working orthochromatic and panchromatic ones. The remainder, the hardworking, non colour sensitive plates, the orthochromatic and panchromatic ones and the infra-red plate, are less frequently used.

The infra-red sensitive emulsion is used for pollen and spores with dark red and reddish and brownish colours. For the remaining emulsions a hardworking plate is recommended for small and faint details. This is impossible in infra-red photography, the choice having more to do with the thickness and colour-density of the object. Good results with small and weak details depend on filter choice (red, dark red or infra-red). If then a harder gradation is desired, the developing can be done in a more concentrated developer.

In order to obtain the greatest possible contrast effect with filters, the filter colour should be in accordance with the colour of the pollen grain. If the latter is clear yellow in colour, the details at high focus become yellow and a yellow filter must be used. The same filter is also used at lower focus for the same details. The colours then appear to be reversed, the details dark and the grain yellow. This rule is also valid for optical sections except for the external details, where a colour compensating filter can be of advantage.

For detail photography:

Pollen and spores	Plate	Filter
Very faint details with pale colour or none	Hardworking non colour sensitive	Bluish green
Very faint details with pale yellow colour	Hardworking orthochromatic	Pale yellow yellow
Clearly visible details with yellow colour	Normal-working orthochromatic	Pale yellow yellow
With very faint details in light orange or red colour	Hardworking panchromatic	Orange red
Clearly visible details in clear orange or red colour	Normal-working panchromatic	Orange red
Details with dark red or reddish black colour	Infra-red sensitive	Red infra-red

In a general survey of pollen and spores with pale colours it may be of advantage to use a compensating filter. If the colour is red, then a green filter is suitable. For pale yellow colour or a blue or bluish green filter should be used.

Sections are not counted as complicated specimens, but it does not mean that no problems can arise at the documentation. For thick sections the emulsion is selected according to the colour or colours shown. In gradations it is as a rule sufficient with the normal-working plates. Filters are sometimes used, when the sections have been too strongly coloured, and in such cases suitable filters would then be weaker or more dense object-colour filters.

For sections with several colours it may be that two obtain the same range or grey colour. With the use of correction filters these can be separated. If the section is blue, green and red in colour and if in photography the blue and

green become alike, they can be separated by a filter, which is in between the two; in this case a weaker or more dense yellow filter.

Thin sections are difficult, but if they are stained, relatively good results may be obtained. For these sections hardworking plates are needed and the filter must always be of a compensating colour. Besides, the hardworking plate should be developed in a concentrated developer, in order for copying to be carried out thereafter.

Diatoms, are, like some of the pollens and spores, counted as complicated objects. Of the four emulsions used the hardworking non colour sensitive plate is for the extremely small and faint details. For other, more contrasted, diatoms there are the normally working orthochromatic and panchromatic plates to choose from. It would be desirable to photograph these difficult objects in as intense short wave light (about 4000A) as possible, but the violet filter on the market lets red light through, so that difficulties in focusing arise. The nearest suitable filter such objects is densely blue or bluish green.

Very small *Diatoms, Coccoliths* and similar objects, cause refraction, so that much faniter colour contrasts (violet + blue) are produced. This colour contrast can be used purely photographically to increase the contrasts. With the use of compensation colour filter (yellow) and a hardworking orthochromatic plate the result often turns out very well. Microscopic *remains of wood,* ground sections of fruits and similar objects usually have such dark colours, that a panchromatic plate with red filter has no effect. The only way to reach the details of these objects is with help of the infra-red sensitive emulsion and I. R. filter. Focusing with such a dark filter must generally be done with quite an open aperture and the highest intensity of light obtainable. The time of exposure is not long, if a fast infra-red sensitive plate is used. It can be compared with a slow panchromatic plate – about 15 DIN. The fast I. R. plate is generally soft, but should a harder gradation be needed, it may be developed in a more concentrated developer.

Epidermal (cuticle) objects are as a rule stained with safranines and the panchromatic emulsion is then the correct one. These objects generally become too darkly and densely stained with the red colour thus necessitating the use of an object colour filter. When an object is too darkly stained it can only be altered in one direction – to more light-coloured. Should it be, on the other hand, given a clear, light colour, there are three possibilities to choose from: without filter, with object filter or compensating filter.

For those working with *replica* the choice of emulsions is easy – generally there are only refraction contrasts present, so that the non colour sensitive emulsion should be used. For small and faint details the hardworking plate should be used and for the rest the normal-working one. A blue or bluish green filter may be used in the wavelength zone, for which the "non colour sensitive plate" is sensitive.

Points to be remembered:

1) For complicated details use optics with the highest possible numerical aperture.
2) The light intensity should be high for complicated objects.
3) The light-field condenser must be equal to the aperture of the objective.
4) Large aperture at focusing.
5) Careful exposure in photographing.
6) The colour of the object or the details.
7) Suitable filter.
8) Correct plate.
9) Correct time of exposure.
10) Always develop at the same temperature.

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ILFORD plate	KODAK filters	LIGHTVALUE																															Deve – lop – ment Time
		*	*	15	*	*	14	*	*	13	*	*	12	*	*	11	*	*	10	*	*	9	*	*	8	*	*	7	*				
Thin Film Half Tone	Blue green									1	1,25	1,75	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40							
Ordinary	Blue green							1	1,25	1,75	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40									
Ortho Half Tone	Yellow					1	1,25	1,75	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											
Chromatic	Blue green												1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40				4min.
	Light blue											1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40					
	Pale yellow									1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40							
	Yellow						1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											
Special Rapid Panchro – matic	Light blue						1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											5min.
	Without						1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											
	Pale yellow					1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											
	Yellow					1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											
	Orange			1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40													
	Pale red		1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40														
	Red	1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40															
KODAK Infra – Red Ex. Rapid	Red						1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40											6min.
	Infra – red										1	1,25	1,5	2	2,5	3	4	5	6	8	10	12	16	20	24	32	40							5min.

Fig. 6. Table of exposure. The table is made up with ocular X 6 and Leitz exposure meter Microsix L. The light value has been selected in the visual eyepiece and the developer used was Ilford ID2.

Plate 1. False and true documentation

Fig. 1. False picture of the surface of a spore. Owing to a too "hard" exposure (small aperture) the underlying details (see Fig. 4) have penetrated. X1500.

Fig. 2. True picture of the same object. Large aperture has been used. X1500.

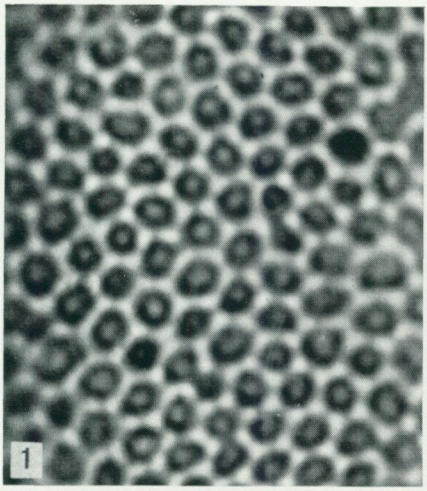
Fig. 3. Illusionary picture from spore. The object's own details (lenses) have produced the picture inside the spore, where there is nothing to be found. X1500.

Fig. 4. Underlying details (the black dots) are arranged in hexagonal formations and have dominated the picture on Fig. 1. X1500.

Fig. 5. False picture of spore. Photographed in X400 and then enlarged X3. $400 \cdot 3 = X1200$.

Fig. 6. The same spore taken with immersion objective and large aperture, resulting in a true picture of the object.

All photographs have been taken with yellow filter and normally working orthochromatic plate.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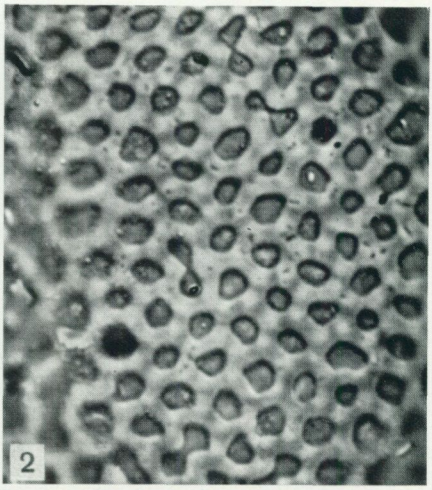
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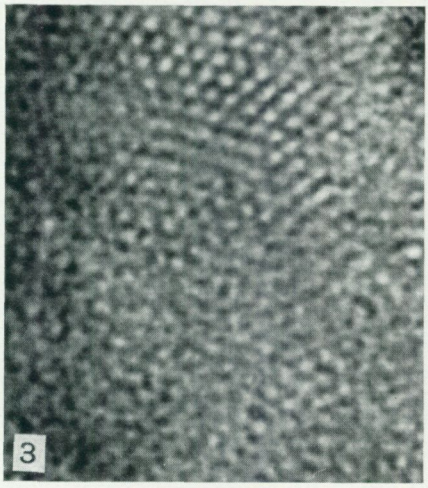
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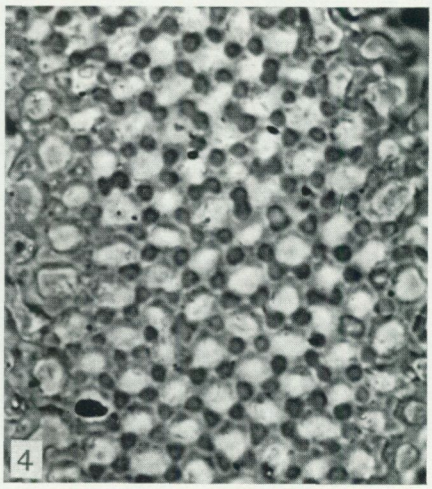
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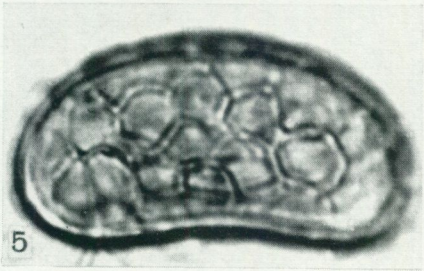
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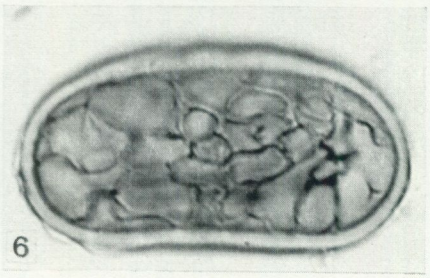

6

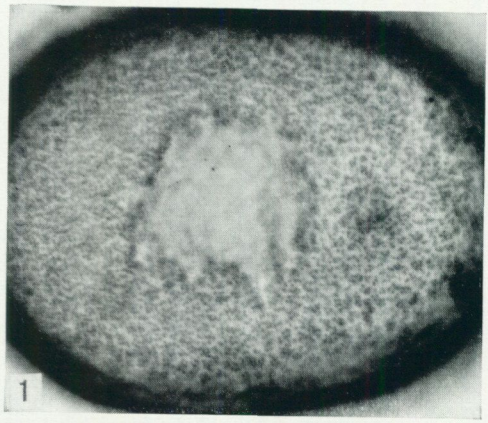
Plate 2. Light and filters

Fig. 1. Grass-pollen with extremely fine details (bordering on what a light-microscope is able to resolve), taken with large aperture and hard-working "non colour sensitive plate" with bluish green filter. X1500.

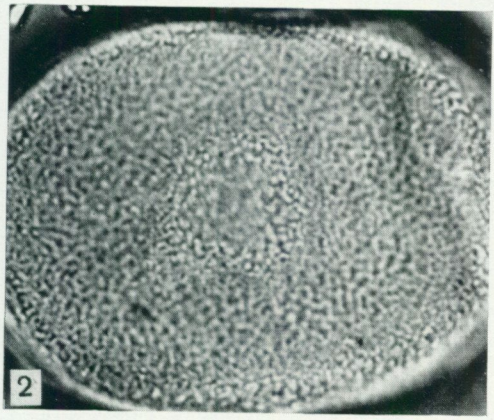
Fig. 2. The same object taken with yellow filter and a normal-working orthochromatic plate. Here the wavelength range has not been able to separate the details. X1500.

Figs. 1 and 2 were taken with the same objective (n.ap. 1.32).

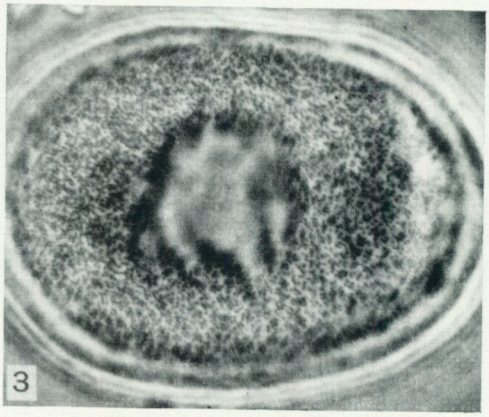
Fig. 3. Specimen as above, but taken with phase contrast equipment. Good contrasts have been obtained, but the resolution (n.ap. 1.10) has not been sufficient for the separation of the in between closest-lying details. Normal-working panchromatic plate has been used. X1500. (Objectives with considerably higher n.ap. are available nowadays on the market.)



1. Blue-green, 4900 to 5100 A.



2. Yellow, 5750 to 5900 A.

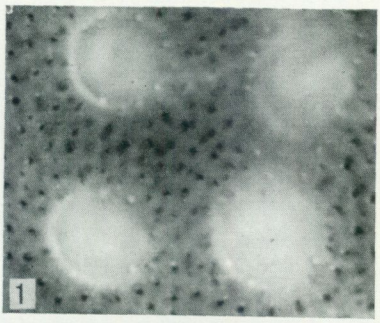


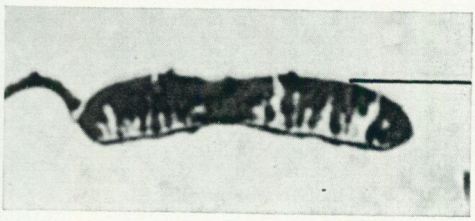
3. Phase contrast, no filter 4000 to 7000 A.

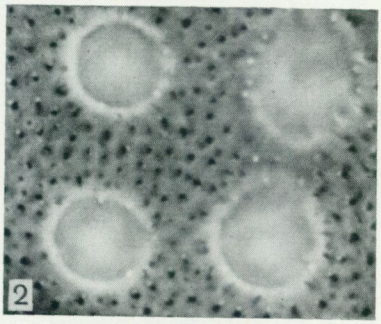
Plate 3. Different appertures

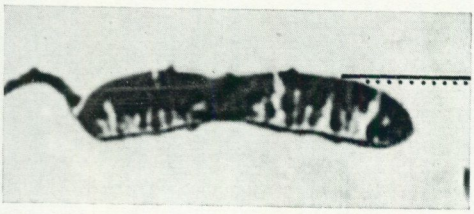
Figs. 1–4. All items on this plate focused in exactly the same position, but with different apertures. Fig. 1 with open, Fig. 2 with somewhat smaller, Fig. 3 with medium and Fig. 4 with very small aperture.
Normal-working orthochromatic plate and yellow filter used. X1500.

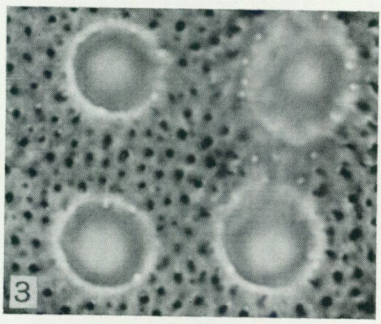
Section: Hardworking orthochromatic plate with yellow filter. Plate developed in a concentrated developer. X2000.

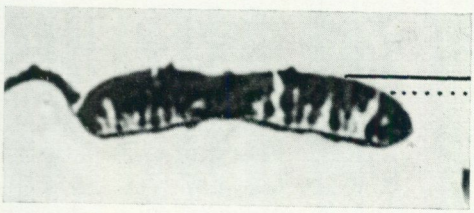


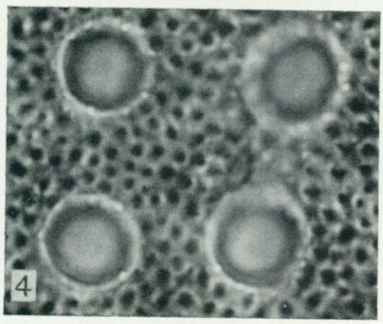












——— = Focus level.
..... = Depth of the field.

Plate 4. Large aperture

Same object as on plate 3. In order to separate the details a large aperture has been used.

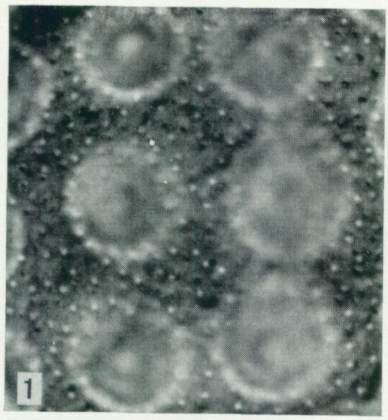
Fig. 1. High focus on the exterior, fine details (white dots). X on the section to the right.

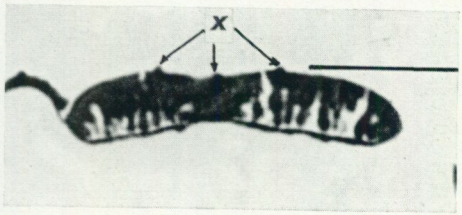
Fig. 2. High focus (dark dots) of the fine pores in the outer wall. Y on the section to the right.

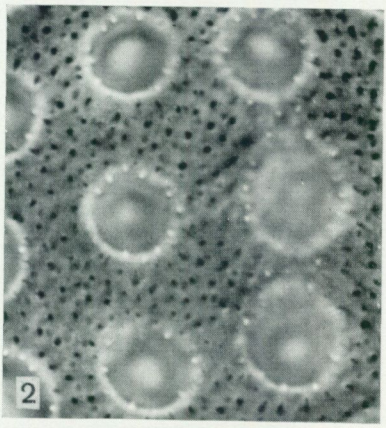
Fig. 3. Low focus of the tap-like details of the wall. Z on the section to the right.

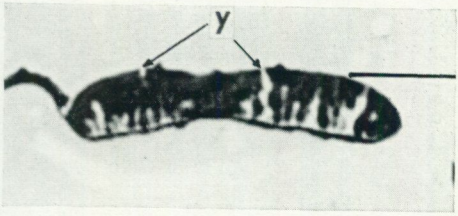
With normal-working orthochromatic plate and yellow filter. X1500.

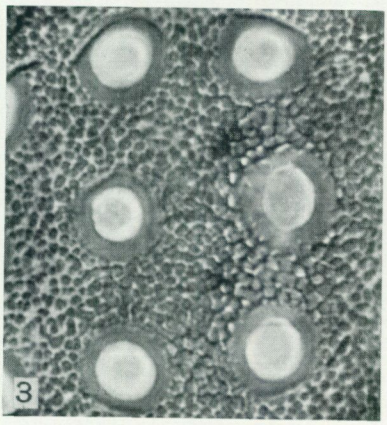
Section: Hardworking orthochromatic plate with yellow filter.
Plate developed in a concentrated developer. X2000.

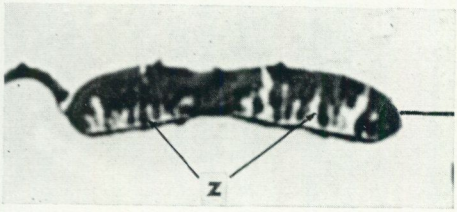












——— = Focus level.

Plate 5. Non colour sensitive plate

Fig. 1. Grass-pollen photographed with hardworking "non colour sensitive" plate and bluish green filter. X1500.

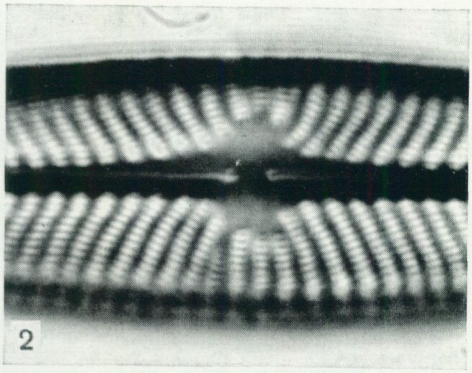
Fig. 2. Diatom taken with hardworking "non colour sensitive" plate and bluish green filter. X3000.

Fig. 3. Epidermis impression (replica) of hair of deer. Normal-working "non colour sensitive" plate and bluish green filter. X250.

Fig. 4. Scale of pike, normal-working "non colour sensitive" plate and bluish green filter. X100.

Fig. 5. Radiolarians (medium focus). Normal-working "non colour sensitive" plate bluish green filter. X1500.


1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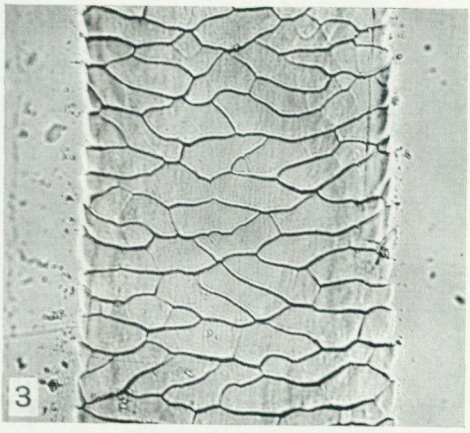
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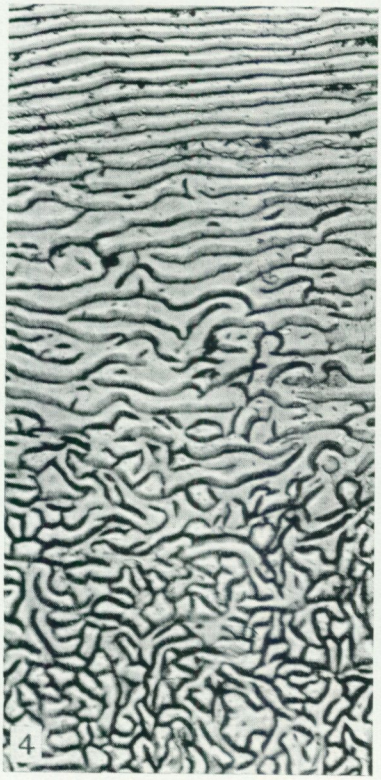
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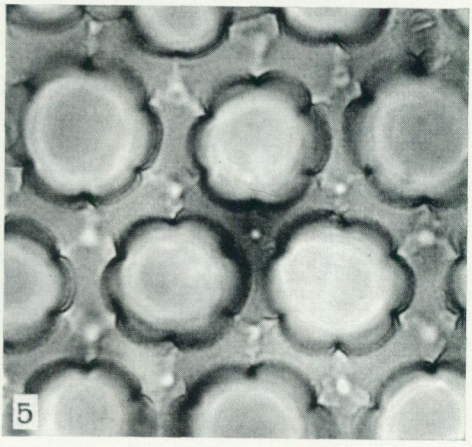
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Plate 6. Orthochromatic plate

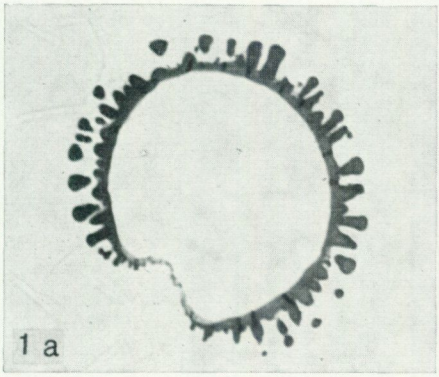
Figs. 1(a) and 1(b). Thin section of pollen.
1(a) with hardworking orthochromatic plate and yellow filter. X1500.
1(b) with phase contrast equipment. Hardworking orthochromatic plate and yellow filter. X1500.

Figs. 2(a) and 2(b). Diatom photographed in high and low focus. Normally working orthochromatic plate and yellow filter. X900.

Fig. 3. Exterior structure of pollen. Normally working orthochromatic plate and yellow filter. X1500.

Figs. 4(a) and 4(b). Diatom photographed in high and low focus. Normally working orthochromatic plate and yellow filter. X2000.

Fig. 5. Detail of foraminifer. Normally working orthochromatic plate and yellow filter. X110.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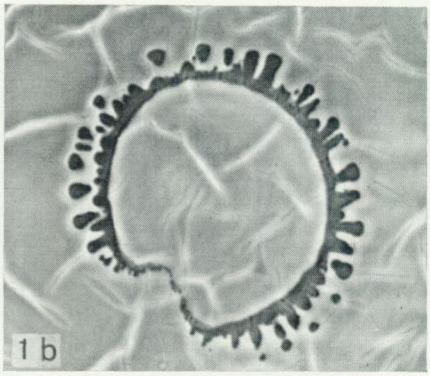
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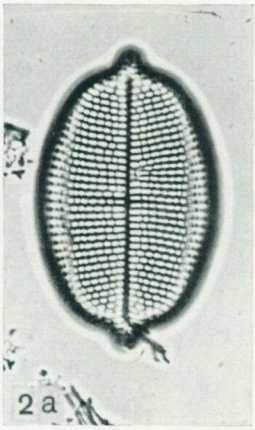
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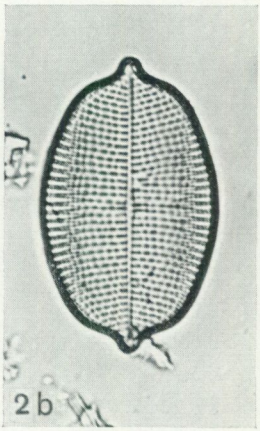
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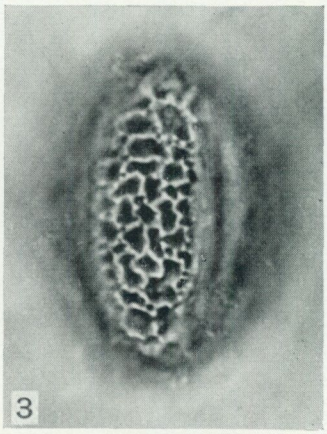
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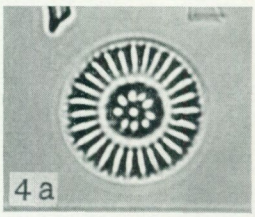
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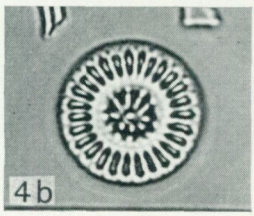
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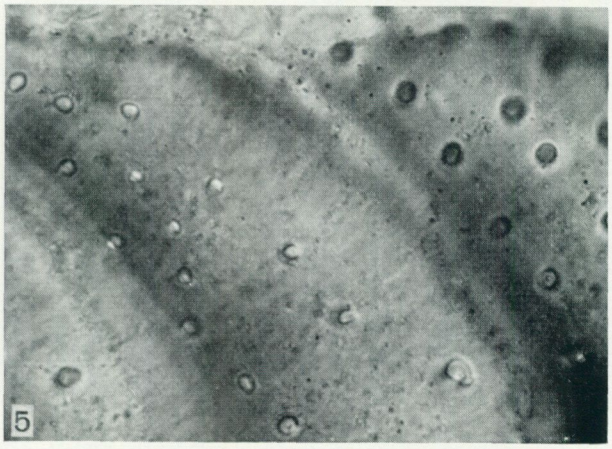
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Plate 7. Panchromatic plate

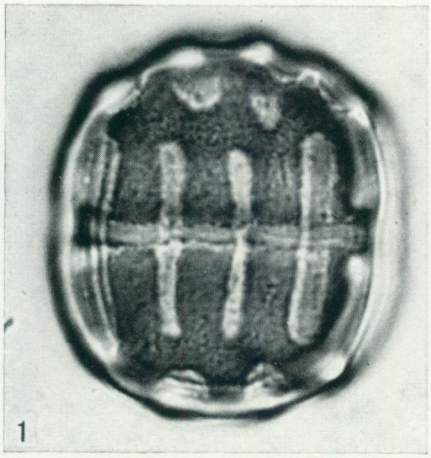
Fig. 1. Pollen photographed with normally working panchromatic plate and orange filter. X1500.

Fig. 2. Diatom. Normally working panchromatic plate and bluish green filter. X300.

Fig. 3. Section of oospore. Normally working panchromatic plate. No filter. X1500.

Fig. 4. Section of fossil wood. Soft working panchromatic plate. No filter. X50.

Fig. 5. Split openings from recent leaf. Normally working panchromatic plate and orange filter. X200.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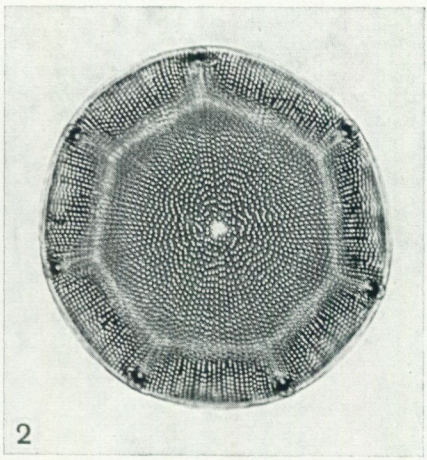
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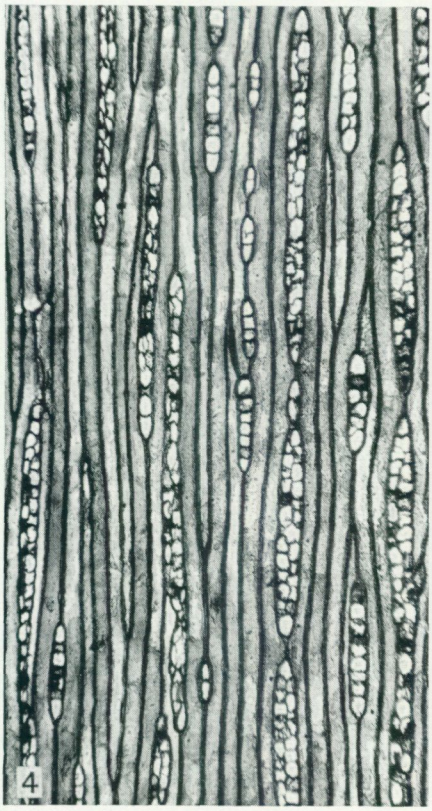
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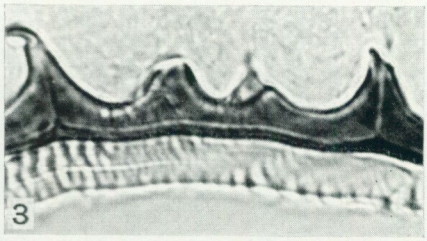
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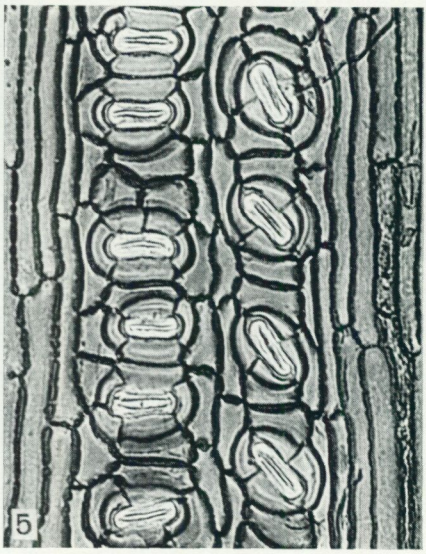
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Plate 8. Infra-red sensitive plate

Figs. 1(a), (b), (c). Recent pollen. (a) and (b) with infra-red sensitive plate and red filter. (c) with panchromatic plate and red filter. X1500.

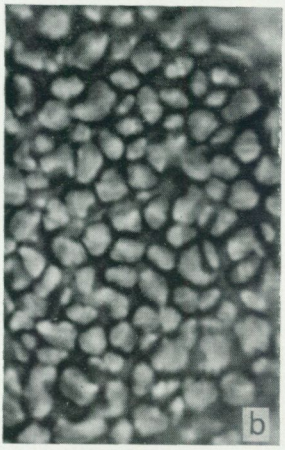
Figs. 2(a) and 2(b). Fossil plankton. 2(a) with infra-red sensitive plate and infra-red filter. 2(b) with panchromatic plate and red filter. X1200.

Figs. 3(a), (b). Fossil wood. (a) with infra-red sensitive plate and infra-red filter, (b) with panchromatic plate and red filter. X400.

Figs. 4(a), (b). Fossil macrospore. (a) with infra-red sensitive plate and infra-red filter. (b) with panchromatic plate and red filter. X80.

Fig. 5. Detail of macrospore (from Fig. 4). Infra-red sensitive plate and infra-red filter. X1500.


a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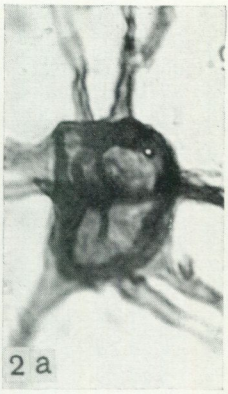
1
c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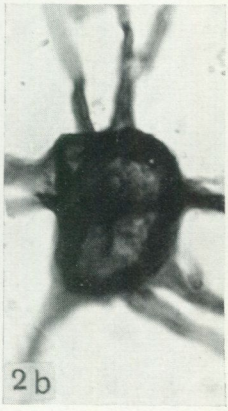
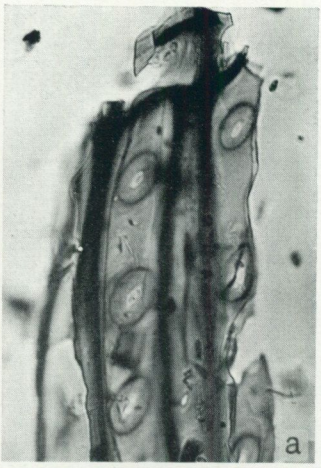

2 b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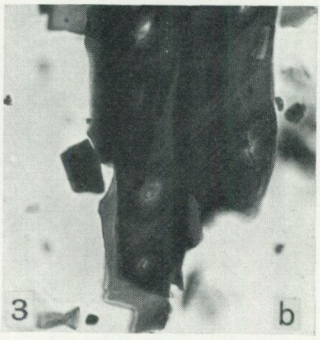
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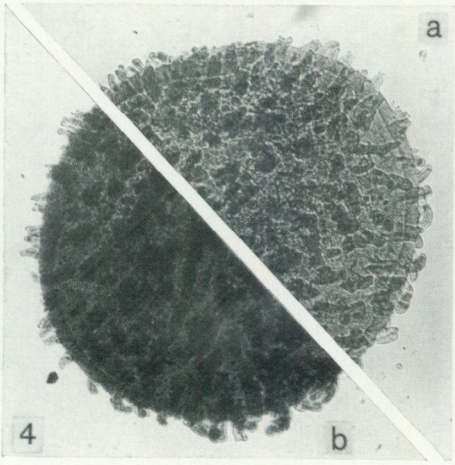
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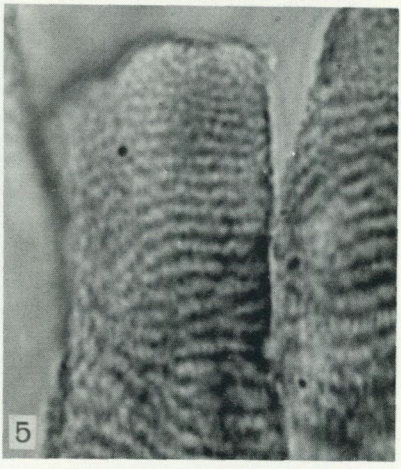
5

PRISKLASS E

Distribution

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