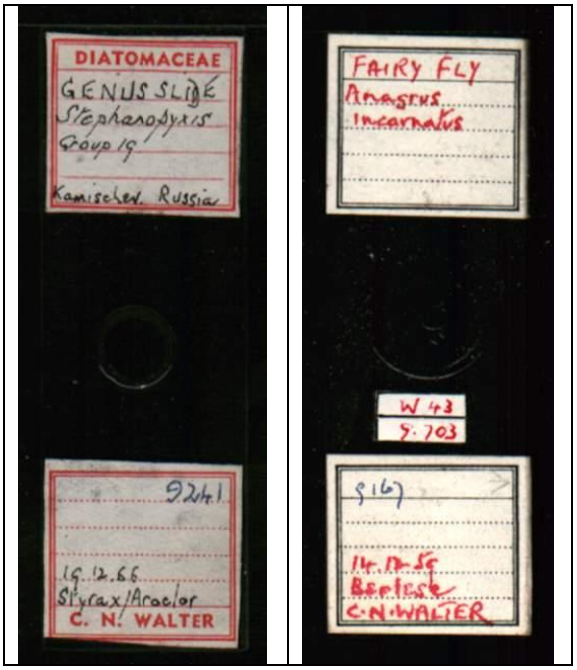
A Diatomist's Vade Mecum

Transcribed and Compiled by Steve Gill

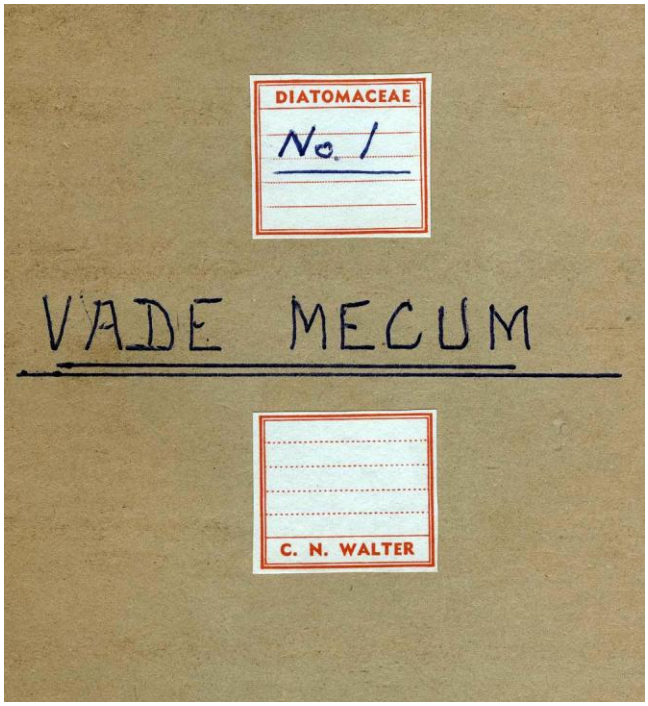


Introduction

The contents of this small volume are the result of a series of happy coincidences in that the material herein ultimately came to be in my possession. However, the various constituents, although originated by Cedric Norman Walter were, subsequent to his demise, scattered amongst other diatomists and amateur microscopists. The *Vade Mecum* was saved from obscurity by Colin Lamb and the Slide Index and a significant number of C. N. Walter's slides acquired by A. V. Dodge. The Postal Microscopical Society notebook arrived separately having been on circuit at one time and subsequently withdrawn a number of years in the past.

To all those individuals and organisations that keep such material safe and ultimately accessible to future diatomists and microscopists I am extremely grateful.

Steve Gill
July 2014



DIATOM MOUNTING EQUIPMENT.

For general use.

- Glass Tubes. 2" x ½"
- Pipettes.
- Distilled Water.
- Cover Glasses.
  $^{3}/_{8}$" for selected. No. 1
  $^{5}/_{8}$" for strews. No.1
- Slides. No.2
- Rinso or Persil for cleaning the glassware and getting it grease free.
- Acid Alcohol 70% MS 99/Acetic acid 1 to get glassware chemical free,
- Canada Balsam and solvent Xylol
- Styrax and solvent Benzene, unless otherwise stated.
- Filter paper. Green's 401. Their 808 is better.
- Filter apparatus.
- Hot Plate.
- Spirit Lamp, Tripod and Asbestos plate
- Ringing table
- Ringing cement
- Rings on underside of mounting slide done in either Indian Ink or Carmine.

Also for Selected Mounts.

- Search and store slides. Ruled with Diamond pencil in 1/8" squares.
- Tracing eraser, for cleaning mounting slide.
- Adhesives. Glycerin Albumen or Acetic Gelatin
- Hypodermic Syringe, to place tiniest drop of distilled water on mounting slide for washing diatom.
- Bristles on sticks. For picking up diatoms. - Cats whisker or pig's eyelash.

MOUNTING PROCEDURE.

SELECTED.

1. PREPARATORY.

Mounting Slides Size No. 2.

To be grease free. Wash new slides in Rinso or Persil.

To be chemical free. Store in a bottle of Acid Alcohol.

For use. Take slide from store bottle, and dry it. Then put rings, Indian Ink, on UNDER side. Central one, very small for the diatom position, and a larger one well away from it for distilled water if diatom needs cleaning.

Cover Glasses. 3/8" Size No. 1.

To be grease free, as above.

To be chemical free, as above. Store in a tube, of acid alcohol.

For use. Take from store tube and dry.

Put guide rings on in Indian Ink, or carmine paint. When rings are dry put in a dry store tube to keep clear of dust.

Mounting Media. To be filtered.

Adhesives. Glycerin Albumen or Acetic Gelatin. Filter through Green's Hyduro 808 filter paper.

Canada Balsam. Filter through Green's Hyduro 808, adding Xylol to thin.

Styrax. Filter through Green's Hyduro 808.

Add solvent to thin it. In oven at 75 degrees centigrade.

Solvents. Through Hyduro 808

Distilled Water ditto

2. PRE-SEARCH.

With clean pipette, take dip from Diatom Stock Tube and put three or four drops into fresh tube with distilled water.

Gently swirl the diatoms in the distilled water

Let stand for them to settle to the bottom.

Change the distilled water and repeat three more times.

3. SEARCH SLIDE.

Should be grease and chemical free. (See No. 1 above). Ruled in numbered squares and marked 'Search` at one end.

With clean pipette a few drops from the Pre-search tube (No. 2 above), to be dropped on the squared part of the slide, with grooves on underside.

Small label one end giving locality, Stock Tube No. etc.

Let stand for the water to evaporate and for the diatoms to dry.

4. SEARCH SLIDE EXAMINATION.

Check over under High Power, by squares, and note any special diatoms by drawing their position in a correspondingly numbered square.

5. STORE SLIDE.

To be grease and chemical free and ruled in numbered squares. Marked Store.

Pick out required diatoms from Search Slide and place on this one, using a numbered square for each Genus, or each Species.

The grooved side under.

6. MOUNTING SLIDE.

Take one with the UNDER guide rings on.

With diamond pen put a reference on the upper or mounting side, to identify, and to know it is the right side up for mounting. The UNDER rings will be rubbed off later.

Upper side. Rub centre with tracing eraser, then put spot of distilled water in centre and again rub hard with eraser to thoroughly clean. Polish with fine cambric cloth.

Examine upper side under 1" D.G.I to see no flaws in area of the mounting circle.

7. ADHESIVE.

With dip rod barely touch the adhesive, then touch centre of mounting slide.

Clean finger tip and rub spot over about 1" square.

Check under microscope for thickness of the adhesive on the mounting slide, and with bristle remove any dirt.

8. DIATOMS.

Put tiny spot Distilled Water in the space provided on the mounting slide, away from adhesive.

Select diatom from search or store slide. If it has any dirt, put it in the Distilled Water on the Mounting Slide, and wash it. Move it out of the Distilled Water to dry.

Pick up diatom, examine it for inner or outer side, and put it down with its inner side on Adhesive at edge of the diatom ring, then push it into desired position.

Check and remove any dirt or bits.

Place slide on Hot Plate for a few minutes for the Adhesive to dry out. Not too hot.

Take slide off and let it cool, placed face downwards in slide rack.

9. CLEARING.

Put drop of appropriate solvent on the diatom. Watch under Micro and see that it clears of air. Check for dirt.

10. MOUNTANT.

When diatom is clear, drain off the solvent, and while the diatom is still wet, put small drop of mountant on.

Examine under Micro for dirt or bubbles in the Mountant and remove.

Let slide stand cold, under cover, for at least 10 minutes. It can remain for hours if required.

For Canada Balsam let stand for 4 days with CB on to let the Xylol evaporate.

11. COVER-GLASS ON.

Take ringed cover-glass from Store Tube.

Check under Micro for condition of ring <u>and that it is uppermost</u>. Also that there are no flaws in the circle area.

With clean paint brush, or cambric cloth remove all dirt from the ringed side.

Examine Mounting Slide under Micro for dirt and remove.

<u>If Styrax.</u>

Handwritten Note: V. Heurck. P81 quotes Peragallo re. Mounting. "Styrax should be heated until it commences to smoke."

Put Mounting Slide on Hot Plate, gentle heat, but increasing, for

7 minutes if solvent Chloroform.

12 minutes if solvent Benzene.

Remove slide to Cold Plate, and let it cool well. The Styrax should become horny.

While on Cold Plate, lay the Cover-glass ring downwards on the Styrax, and place the slide on Hot Plate until Styrax runs to edges of the Cover-glass.

See the Cover-glass Ring and the slide under-ring are concentric.

Remove slide and examine it at once under Micro to see the diatom is central to the Cover-glass Guide Ring. Very gently push Cover-glass with a needle to do this. If Cover-glass is set too firm, replace on Hot Plate to soften the mountant and check again immediately under Micro before mountant hardens.

If Mountant looks too deep, run a swab·of solvent round edge on one side and put back on Hot Plate. This will cause excess to exude, then repeat with the other side to level the Cover-glass.

If Canada Balsam

Place Mounting Slide on Cold Plate and put Cover-glass on, ring downwards.

If Canada Balsam does not run to the edges try very gentle heat, or infill.

Check diatom is central to Guide Ring on Cover-Glass.

Place slide in oven for 24 hours at 62 degrees centigrade. If Balsam is not brittle put back for further heating.

12. CLEANING.

If Styrax

The Cover-glass should be set hard and the excess horny.

With forceps dip tiny cotton wool swab in solvent and carefully remove excess mountant. In same way with fresh swab wash the top of the Cover-glass.

Examine slide again to see the diatom is central to the Cover-glass ring. Heat and adjust if necessary and remove excess as before.

If Canada Balsam.

The excess should be brittle and can be scraped away with a safety razor.

15. RINGING.

Put on first ring of cement. Let stand for 24 hours.

With forceps soak tiny cotton wool swab in Rinso solution and carefully clean the Cover-glass and the whole of the front and back of the slide, and remove the under-rings.

Put on second cement ring.

14. LABEL.

With fullest information.

MOUNTING PROCEDURE.
STREWN

1. PREPARATORY.

Mounting Slides.

Size No. 2.

To be grease and chemical free, as for Selected Mounts.

For use. Under - ring the Mounting Slide for $^{5}/_{8}$"or other size.

Cover-glasses.

Size No. 1. $^{5}/_{8}$"

To be grease and chemical free and NOT ringed.

2. PRELIMINARY.

Clean diatoms in tube as for Selected, Pre-search.

3. DIATOMS.

Take a spare slide, put a spot of moisture on it, and put the mounting cover-glass on it so that it does not move. Two, or even three cover-glasses can go on the one side provided they are the same material. Mark slide to identify material.

With a clean pipette and using the bulb gently swirl up the diatoms in the Distilled Water Tube.

Draw up a small quantity while they are in suspension.

Breathe on the Cover-glass and let a drop fall from the pipette at about 1" height. A $^{5}/_{8}$" Cover-glass requires two drops. See that the drops do not run over the edge, or will get too thin a spread.

Put slide on Hot Plate to dry out the Distilled Water on the Cover-glass. A few minutes will do this. Heat also helps the diatoms to adhere to the C G.

Examine C G. under Micro when dry, to see spread is not too thin, and also to remove dirt and hairs.

4. MOUNTING.

Mounting Slide grease and chemical free, under-ringed as No. 1 above for size of Cover-glass.

With diamond pen put reference on the mounting side of the slide.

Drop of mountant on the slide in centre of the circle, and let stand for time appropriate to the mountant.

Put slide with the Cover-glass of diatoms on one end of it on Hot Plate and follow procedure in Selected No. 11 Cover-glass on.

5. CLEANING & RINGING.

Follow Selected 12 and 13.

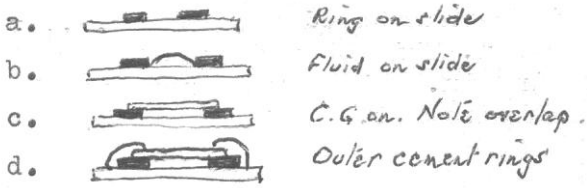
6. Label.

Appropriate details.

FLUID MOUNTS.

Slide grease and chemical free, cover-glass also.

1. Ring of cement wide enough to ensure it will overlap the cover-glass



2. The material should be in 2% Formaldehyde.
3. When cement ring is just tacky
4. Swirl Diatoms into suspension
5. Drop on from pipette like b. above
6. Put C.G. on diagonal method, or better still as detailed below.
7. See it is completely inside the cement ring
8. Very gently press edges into the cement so the cement does not run inside.
9. Remove excess fluid with strips of filter paper. Opposite sides at same time:

10. Examine under Prior to see the C.G. is bedded in the cement ring and no bubbles.
11. See edges are dry and put thick cement ring on.
12. Add two more cement rings at intervals of 24 hours.

Putting C.G. on. Done diagonally as above may cause too much fluid to go out on one side, taking many diatoms. Take a small glass tube with less than $^{5}/_{8}$" base, damp base with saliva, press it on C.G. and it will pick it up. Then gently lower the tube so that the C.G. comes down right in centre of the fluid, gently releasing the C.G. with forceps. lf C.G. is kept level, excess fluid will go out evenly all round.

Another method for this form of mounting is to make the original mounting ring with paraffin wax. It, of course, hardens immediately, but after C.G. is on, heat a needle or something similar and run it round the edge of the C.G., very gently pressing down. This should melt the wax enough for G.G. to sink in. The wax is not miscible with water or the cement.

DRY MOUNTS.

E. Marson's Method.

1. Ring of Brown Cement. Outer edge of the ring to be level with the outer edge of C.G., i.e. the ring on the turntable, not beyond it.
2. Let stand until just tacky
3. Specimen should be on C.G.
4. Invert C.G. on to the ring and gently press down round edge with forceps.
5. Later ring with cement.

Note. The ring under No 1 above wants to be deep enough for the thickness of the diatom.

Per S.H. Meakin.

1. Gold size ring on slide to be quite hard.
2. Diatoms mounted on C.G.
3. Warm slide enough to soften the Gold Size
4. Make C.G. very hot
5. Put C.G. on and cool as quickly as possible
6. For selected specimens Guide Ring should be on the Slide

Will not get "dewing" if C.G. very hot and the Gold Size quite hard.

A method I have used for Strews.

1. Diatoms on the CG as usual.
2. Clean slide and a $^{5}/_{8}$" ring of 50% Murrayite with 00 Brush. Thin.
3. CG on at once lightly
4. Centre it to the Murrayite Ring and gently press down with point of needle.
5. Place another slide carefully over it and hold together firmly with left hand so will not move.
6. Press down CG all round with Right Hand.
7. Ring with normal Murrayite (or Cement)

Don't know where I got this from but it works.

MULTI-MOUNT.

1. Pick out diatoms from Search to Store Slide and wash them in Distilled Water.
2. Use each square on the Store slide for a Genus or a species.
3. Under Research Microscope, examine the Store Slide and draw a sketch of each square giving the position of the diatoms in it

4. On the sketch, number each diatom serially.
5 Prepare a list of numbers, seriatim
6. Place slide on Service I, reversing the way to when on Research and the lay-out of the diatoms in each square will appear the same as on sketch
7. Under high power identify each diatom and note it on the numerical list, or strike out any which will not be good enough.
8. When list has been completed, prepare another list by Genera and Species, recording against each the number and the square in which it can be found on the Store Slide.
9. Then decide which to mount, ignoring those which are damaged or too dirty.
10. Prepare a plan showing the order or the columns in which diatoms to be mounted.
11. Use fresh Adhesive, Glycerin Albumen.
12. Settle position of the centre column. Put a distinctive diatom at the top centre as a guide for the rest
13. On picking up from Store Slide, examine each diatom to make sure which is inside and outside, before placing on mounting slide under Research.
14 . Put a few diatoms on the mounting slide approximately in position. Move them well on the adhesive.
15. Transfer slide to the Service II and put diatoms in position with the Mechanical Finger
16. If movement of diatoms is slow or sticky, breathe gently on the adhesive.
17. If any diatoms do not respond, or are awkward to move, or stick and then jerk forward, remove them and replace with others.
18. Get the first column completed
19. Do the remaining columns on each side of it.
20. When all are completed, hold the slide up over the Hot Plate and slowly lower it so that heat is gradual and not too hot when on the Plate. Let it stand for a half hour.
21. When slide is cool, clear in Xylol.
22. A drop of Canada Balsam on to cover the diatoms.
23. Let this stand cold, under cover, for at least 48 hours.
24. Warm slide
25. Heat cover glass
26. Tiny drop of Canada Balsam, size pins head, in centre of Cover Glass.
27. Cover glass on.
28 Warm slide and Canada Balsam will run to edges.
29. Centre the cover glass and see it is level.
30. Let it stand for a few hours.
31. Check centration.
32. Into oven
    - 45°C 12 hours
    - 50°C 12 hours
    - 62°C 12 hours

Of course, the usual points will be watched as j for Selected Mounts re dirt etc.
Use Canada Balsam as the reaction of the Xylol and the Canada Balsam is least likely to dislodge a diatom. Styrax is much more violent. Also the diatoms are variable in size and depth. The purpose of these mounts is to show type for a locality or a Genus, and serves that purpose. Resolution of fine detail is better done on a single selected mount when appropriate mountant for that particular diatom can be used,

GENUS SLIDES.

These can be:

(a) A group of one each of several species of the same Genus.
(b) A group of several of each species of a Genus.

The diatoms should be first transferred from Search to Store slide, being grouped on the latter by species.
From there, the individual species can be picked out for (a) above.
For (b) above several of each species are required, and should, as far as possible, be grouped together on the Genus Slide. This may want planning beforehand. See Multi Mount procedure, as previously described.
The diatoms will be arranged in columns or rows for quick and easy reference.

TYPE SLIDES.

There are three kinds:-

(a) A group of one each of many species of Diatoms irrespective of locality, or habitus, and grouped on the slide in genera.
(b) The same, only all from one locality, to indicate what can be found in that locality.
(c) A group of species of Diatom from one locality, but the number of each species on the slide related to their preponderance in the locality. The aim is to show the dominant form (large number), prevalent (a few) and scarce (only one).

The diatoms will be arranged in columns or rows for quick and easy reference.
Follow the Multi-mount procedure as previously described.

COMPARISON MOUNTS.

These are for making comparisons of Mountants etc. on the same slide. Prepared at the same time and mounted as like each other in detail as possible.

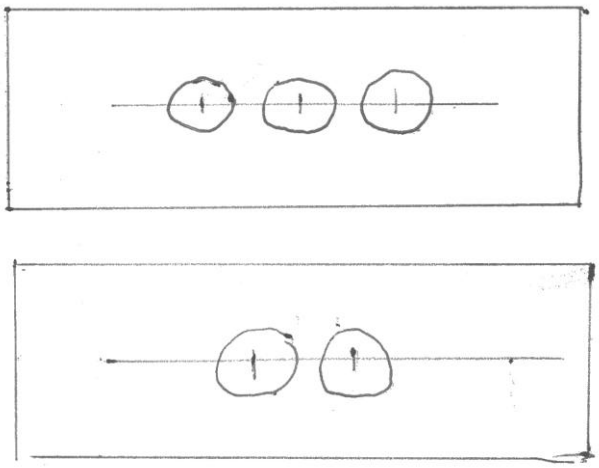
Triple.

Put cleaned slide in Slide holder.
with Biro, put three marks on it from left to right at 1", 1½" and 2" on a centre line.
Put slide on ringing table, centre each mark and make a $^{3}/_{8}$"
See below
Rings $^{3}/_{8}$" with gap between each of ⅛"

DOUBLE.

Similar procedure. Centre line, then make a mark on it 1¼" from each end. Make $^{3}/_{8}$" rings on turn table, and they will be ⅛" apart.
See below.

The above, are of course, under-rings. Follow the mounting for Selected or Strewn, as required.



FILTERING.

The best Filter Paper for diatom work is Green's Hyduro 808, obtainable from

J. Barcham Green Ltd,
Hayle Mill,
Maidstone, Kent.

Their 401 can be used, it is a general purpose filter paper, but is not so fine as 808. The rate of filtration of 808 is very slow and therefore retains the finest precipitates.

I use a small plastic funnel obtainable from Camera Shops, about 1½" diameter, separate one kept for each medium, and wash it each time before using.

Fold the circular filter paper four times then unfold and fit into funnel.

I cut off most of the spout of the funnel as it is too long to go into a 1 oz. bottle.

The adhesives filter fairly quickly, but the styrax is very slow. Add more solvent and use heat. I put it in the Thermostatic Oven at 75 degrees C.

Better to do a little at a time, I think it keeps better.

R.I.F says do not use a glass funnel. He makes a loop at top of a piece of upright wire, the loop being right size to take the folded filter paper. I have one like this, but use the funnel because the funnel rests in the 1 oz. bottle and dirt cannot get in during process.

LABELS.

I ordered 2,000 from Flatters and Garnett, printed "Diatomaceae" for top label, and "C.N. Walter" for the bottom label.

Each mount has my reference on it, as mentioned in the Mounting Procedure. I now cut pieces of paper the size of a slide, and write on the top and bottom ends of each the information to go on the label, and note the reference on it as well.

Immediately after putting the labels on the slide, procedure below, I pencil the reference lightly on the bottom label, then place the paper slide with the mount over it in a slide tray to let the labels stand for a few hours to dry on. Then put the details on the labels using either Indian Ink or Biro.

To write as small as possible it can be done under the Prior Microscope at X7.

<u>Label procedure:-</u>

Trim edges of label.
See the two ends of the slide have been cleaned with Rinso, and polish them.
Wet the ends of the slide and also wet the gum on the label.
Place label accurately on and press down by putting a clean piece of paper over it and gently smoothing out
Information on, as detailed above.

REFERENCES ON SLIDE.

It is important that when commencing a mount some reference should be put on the mounting slide to enable the mount to be identified and all information given when it comes to labelling.

I keep a Diary, and in that record full details relating to each mount as I proceed. The pages are numbered, so with a diamond pen I put the page number on one end of the mounting slide - on the upper side, i.e. the one on which the diatom is to be mounted. If several mounts relate to the one page, then they are recorded in the Diary Page as, e.g. 50.1,, 50.2, 50.3, etc with any notes against each specific to that mount. These numbers then go on the slide. The number is later covered by the label.
This method is also useful to determine at any stage that the mount is right side up, as the numbers would appear in reverse if not so.

CENTRE OF SLIDE ON SERVICE II.

Vernier readings are 88 X 31, and the centre of the field of view is the centre point of the slide.

PRESERVING MATERIAL.

Keep in tubes, one for each locality and appropriately labelled. Preservative is:

Distilled Water 95%
Phenol (Crystals), 5%

Filter mixture before using. Every year or two the preservative should be decanted and replaced with fresh.

CLEANING SLIDES.

Grease Free.

Take fresh box of slides; put them one at a time into a bowl of warm Rinso, or Persil and let stand for an hour.

Take out one at a time, lay on a piece of Kleenex and fold over. When the Kleenex is dry the batch can go back into the slide box, still in the Kleenex, and marked "Degreased".

Chemical Free.

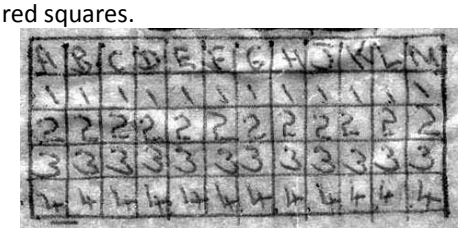
4 oz. bottle containing Acid Alcohol (see below). Take degreased slides and put into the bottle where stored until wanted. When taken out for use let them dry without rubbing.

ACID ALCOHOL.

Per Gray.	**Per E.Marson.**
Acetic acid 1%	Hydrochloric Acid ½ ml
70% M.S. 99%	75% M.S. 100 ml.

RULING SEARCH & STORE SLIDES.

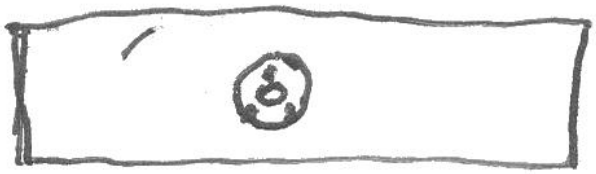
Rule a piece of paper size of a slide B" X I"
⅛" squares. There will be 48 numbered squares.



Put this paper slip in slide rack and the cleaned slide on it. Using another slide to provide an edge, and pressing it down firmly rule all lines with diamond pen.
Then, on the same side insert the letters and numbers as in above diagram. Do this on dark ground.
The other side of the slide will be smooth and the one for use, and letters and numbers will appear right way round. With Gurr's Glass Ink, at one end, put "SEARCH" or “STORE". The reading of these words will ensure slide is right way up when diatoms are put on it.
Clean with Rinso and store in Acid Alcohol.

SUPPORTS FOR COVER GLASS.

I have a quantity of these but hardly ever use them.
The difficulty is to position them so that the CG can be centred with its guide ring in relation to the diatom, and the supports inside the edges of the cover glass. Only way to use the supports is to have the guide ring on the slide with the diatom fixed in the centre of it. Can then put supports on well inside the CG edges thus:



They should be placed in position on the adhesive before it is heated dry. They are useful for a very deep diatom when CG could not rest level. S.H. Meakin never used them for the reasons I have given above and depended on sufficient depth of mountant.

RINGING & CEMENT.

I use Brown Cement, sold by Flatters & Garnett. This is Shellac. To make it Black, it could be added, as can be seen from the formulae below.
Use a No. 1. sable hair brush. Small blob on tip, raise brush vertical so that the Cement just goes a little way down the sides. Use brush at an acute angle

thus 

not 

Turntable, brush, hand, forearm, and shoulder all in same axis.
Two applications at intervals of 24 hours.
Trim the outer edge and the inner edge of the cement with point of a penknife. Turntable running fast.
Formulae:-
Per S.H.Meakin

Ordinary Shellac
Dissolve in Goodrich's Methylated Spirit. (Sold in shops)
Then add plenty of Boots Black Spirit Dye.
Let stand for a week or so, shaking twice daily.
Then filter through muslin. May have to squeeze the Shellac through.

Per E.D.Evens

(a) [Shellac 10 gm
Castor Oil 1 cc
100% M.S. 12 cc

Lamp Black 1 gm (Carbon black)] (b)

Use (a) for the first ring and (b) for the final ring.

MURRAYITE.

Flatters & Garnett do not recommend this for Diatom mounts as they say it is not impervious to Immersion Oil.

GUIDE RINGS ON COVER GLASS.

On a plain slide, put tiny spot of saliva in centre, and place a cleaned CG on it and gently press it down. This will keep the GG in position. Put on ringing table and centre the CG to the $^{3}/_{8}$" ring on the ringing table.
Use a ROTRING VARIANT PEN 0.2 sold at Drawing Office suppliers. Use the holder arm on the turntable. It will give excellent rings, any diameter, and contains Pelikan Black Indian Ink. (Use filtered Ink)
Another way is to use a mapping pen (see later). Put small drop of the Indian Ink in a watch glass or paint tray, and dip tip of pen in it. Use it as above with the table holder. It is not so satisfactory as the Rotring.
It is wiser to do a good number, say 100 at a time while one has got the knack of it easily; the first two or three may not be perfect. When they are dry store in a tube.
(Handwritten Note: Carmine paint is a good alternative. Use a 00 Sable Hair Brush, after removing all but 3-4 hairs in centre.)

BRISTLES
ENTOMOLOGICAL PINS

Pig's Eyelash in a holder is very good; Cat's Whisker also. I am using a holder which belonged to the late Morley Jones.
Entomological Pins are also good, if in a light holder. The point can be fined or thickened on Wet/Dry Emery Paper. See below.
Pins are obtainable from

Janson & Sons,
44, Great Russell St. W.C.

Size 000·and 0 are the two smallest sizes and have the finest points. They are stainless steel and 2/6d per packet. Next sizes, going upwards, are 1, 2, 3, 4.

WET OR DRY EMERY PAPER.

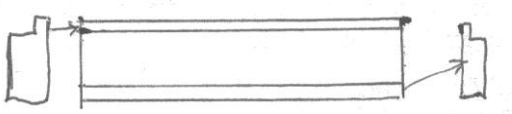
Called, WET OR DRY TRI-M-ITE PAPER, two kinds one is finer than the other.

W. 280. A. Soft Back
W. 400. A. Soft Back

Obtainable at Garage.
Very useful for shaping the point of a bristle or a pin.

SLIDE RACK.

To hold a batch of 12 slides, after heating diatoms on the adhesive, so that the side the diatoms are on face downwards. Rectangular, open in centre-and a ledge along each side for the ends of the slide to rest on. Keeps dust off the mount.



FORMALIN.

This means diluting 40% Formaldehyde which is how it is sold, in Distilled Water.
Proportions as follows:-

Grade required	40% Formalin	Distilled Water
2%	5%	95%
3%	7½%	92½%
5%	12½%	87½%
8%	20%	80%
10%	25%	75%

TO UNMOUNT.

Under Prior, remove cement ring with hot razor blade, working from inside to outside. Then go round edges with swab in the mountant solvent. Gentle heat on Hot Plate should soften the mountant and be possible to gently raise the edge of the cover glass with razor blade.
(Per Caballero y Bellido)

MAXIMUM NUMBER OF DIATOMS ON A COVER GLASS.

According to Caballero y Bellido a CG of 16 mm diam., $^{5}/_{8}$", will take 4,000.

SLIDE THICKNESS.

Use No. 2 Slides,

a. Because Service Microscope is adjusted for No. 2
b. Because No. 1 is likely to break should it get too hot on Hot Plate

CENTIGRADE FAHRENHEIT.

Boiling point Water. 212°F. 100°C.
To work out Fahrenheit to Centigrade.
Deduct 32 and multiply by $^{5}/_{9}$:-

212 – 32 = 180
180 X $^{5}/_{9}$ = 100°C

Centigrade to Fahrenheit
Multiply by $^{9}/_{5}$ and add 32:-

100 X $^{9}/_{5}$ = 180
180 plus 32 = 212°F

BIRO RINGS.

For under-ringing a mounting slide, a Biro BIC is useful, and for hand-mounting on the Research Microscope proceed as follows: -
$^{3}/_{8}$ ring.
Place slide in slide holder and rule a central horizontal line across the circle, and then a vertical. This will be O.K. for large diatoms, but not quite so satisfactory for small ones, because the Biro line, seen under the Microscope, is double.

WATERPROOF INK.
INDIAN INK.

Pelikan Black is best, sold in Drawing Office Shops.

GURR'S GLASS INK.

From George Gurr Ltd. Red. This is fairly permanent and takes a good deal of rubbing off, whereas Indian Ink and Carmine come off at once.
Shake the bottle up well before use and stir it.
Use a mapping pen. Put small drop on watch glass or paint tray and dip tip of pen in it. If dip pen in bottle can get it too thick.

MAPPING PENS.

Gillotts Crow Quill 659 This is best.
Kingsley, Hinks, Wills & Co. 2788
Brandauer. No. 311

DIATOMS - ADHESIVES. 21/6/65

Glycerin Albumen.
When I took up mounting diatoms I only had a tube of Glycerin Albumen which I used for mounting sections. It worked all right for diatoms but had not been filtered and left bits of dirt on the slide.
Mayer's Glycerin Albumen.
F. B. Taylor advocates this as an adhesive for diatoms. I bought some from T. Gerrard & Co. Ltd, liquid form, and filter it before use. It is an excellent adhesive but one has to check for tiny bits of dirt which occasionally appear and remove them before mounting the diatom: Unlike some of the other adhesives it does not dry for several days and is most useful for doing a multi-mount which may take three or four days to do. One does not have to keep breathing on the slide.
Gum Tragacanth.
The late Morley-Jones of the Quekett was a fine diatomist and used this. The formula is:-

Gum Tragacanth powder 1 cc
Distilled Water 99 cc
When dissolved add Phenol · · -.3 cc

Filter before use.
I find this O.K. for single selected mounts but do not use it for multi-mounts because of having to constantly breathe on the slide to move the diatoms into position.

Dextrin.

This was used by S.H. Meakin and also to-day by R.I. Firth. Meakin gave his formula in his article in "The Microscope" Vol. 13 p 69 as:-

Dextrin flour ½·oz (15cc)
Water ¼ ox (7½cc)
Dissolve in water bath
Add Glycerol 1½ ozs (50cc)
When dissolved and cold add
Phenol 3% (2½cc)

I tried to make this up but probably got the quantities wrong as the resultant fluid was not satisfactory. Diatoms would not stay put, anyhow I had the G.A. and G.T.

Have just looked up Adhesives in Peter Gray's "The Microtomist's Formulary and Guide" and on page 661 he gives:

Meakin Formula:

Water 12.5
Dextrin 25
Glycerol 75
Phenol 3

Dissolve Dextrin in water with heat. Add other ingredients. Filter before use.

I can understand these proportions so will make some up and see how I get on. It may not be any better than Mayer's G.A., because R.I. Firth said after putting on the smear of Dextrin examine the slide for bits of dirt and remove them.

Gelatine Acetic.

A Quekett member gave me the following formula:-

Gelatine powder 1%
Acetic Acid 99%

Filter before use.

Smear dries at once on slide and have to keep breathing on it. Diatoms did not always keep in place.

DEXTRIN
PREPARATION.

R.I. Firth's Method.

Dissolve a little Dextrin flour in Distilled Water (which has been filtered Hyduro 808).
Add a little 2% Formalin.
After it has dissolved apply gentle heat for two or three days.
Cover tube or jar while doing this with filter paper.
Now add about half the quantity of Glycerine.
Make a perfect mix with a glass stirring rod.
Pass the mix through a medium filter (Greens 401) straight into storage jar. See jar is clean first.

Bellido's adhesive.

In the P.M.S. Library is a book called "Technique of Systematic Microscopical Preparations" by Ernesto Caballero y Bellido. Original was in Spanish but this English translation has been written out by Dr. Spence and given to the P.M.S. Caballero specialised in large arrangements of diatoms and tells in this book how he did it all. Not of any use to me, because he had a specially made stage, all covered in, so that the work was done in an air tight compartment. He used to hold courses of instruction in his method - but that was 40-50 years ago and in Spain. His formula for his adhesive was:-

Best Gelatine 1.5 g
Distilled water 12 ml
Glacial Acetic 11.8 ml
100% Alcohol 2.5 ml

Filter three or four times before use. It must be used fresh so wanted frequent replacement.

I tried making this up but it would not hold one diatom on a mount, let alone a group. I may not have prepared it properly and was using it under different conditions. His adhesive supply was in the air tight stage.

Incidentally; for mountant he used Monobromonapthalene of which I know nothing.

Since writing the above I have accidentally come across Caballero's adhesive formula in another part of Peter Gray's Formulary. viz;-

Water 48
Acetic acid 50
Abs Alc 2

Gelatin 6

Soak Gelatin in water overnight. Melt at 90°C and add other ingredients.
Also instructions of how applied to slide and used.
I will try this.

ADHESIVES.

Acetic Gelatine.

As advertised by Flatters & Garnett for mounting Diatoms. It wants filtering. Found a single diatom would remain in position but results variable when mounting several as a group.

In reading an old copy of the R.M.S. Journal I came across the following, which should overcome the above mentioned difficulty:-

Aqueous solution 1% Gelatine, containing 1 drop Formalin per 10 cc, made up and filtered through fine silk.

One drop applied to slide centre. Surplus gently poured off and resulting gelatine film allowed to set under watch glass. Put Diatom on it, in position; carefully breathe on it. In a few seconds the gelatine sets firmly again and the, diatom permanently held. Do this each time a diatom is placed in position.

Dextrin.

S.H. Meakin stated that he found most of the adhesives, including Glycerin Albumen, would not keep more than three months, when they went bad.

He found his Dextrin mixture. quoted above, the best, and was using some 10 or 12 years old as good as ever., But he said the Dextrin he used was called British Gum and is cream coloured; he added that the white powder obtained from London chemists is no good.

DIATOMS - MOUNTANTS. 21/8/65

Canada Balsam. R.I. 1.526

Can get good results for the more robust types of diatom. I use Cole's Exposure Method, and after placing the Balsam on the diatom,·I let it stand, under cover, for three or four days. This gives the Balsam chance to thoroughly permeate the diatoms and for the Xylol to evaporate. The Balsam hardens. Then tiny drop of C.B. on cover-glass, invert it on to the slide and the C.B. runs nicely to edges. After that, I place it in a Thermostatic Oven at 60 deg. C. for 36-48 hours.

I use C.B. for multi-mounts, because the reaction of the Xylol for clearing and the C.B. when put on, is very much less than with Benzene and Styrax; Styrax is quite violent for a few seconds. Much less chance of a diatom being pulled out of place.

A multi-mount done this way will show variation in brilliance between the same species, which I think supports my view that there is much individual difference in robustness and depths of marking in the same species. One diatom may resolve everything in C.B. and another of the same species wants a higher R.I.

Styrax. R.I. 1.583

The most commonly used mountant since Van Heurck introduced it eighty years ago. It has stood the test of time; but it depends on how it is prepared. The best diatom mounters (down to Meakin, and to R. I. Firth today) make up their own Styrax, giving considerable time and care to it. The batches vary and they seek for the lucky one, which may provide enough for all their future requirements. R.I. Firth told me he was lucky in 1955, the best Styrax he had ever made and, fortunately, enough to last him out -and he is a professional mounter.

I am not competent to make the preparation and have to purchase mine ready made. The best I seem to have obtained is from George Gurr Ltd.

(See RI. Firth method later)

Clearax. R.I. 1.666

This is a proprietary of George Gurr Ltd. Have tried it but not very keen on it. Bubbles are a trouble. Two or three members of the Quekett use Clearax, but they do not worry too much about the bubble as long as it is not in the field of view of the diatom. Not my idea. I like a perfectly clear mount.

Hymount. R.I. 1.666

This is a proprietary of Edward Gurr Ltd and is their contra to Clearax. I have not tried it yet, but I think J. Grover of the P.M.S. has.

Brigger's Styrax. R.I. 1.642

Obtained a small quantity of this at the Quekett one night, but have not tried it yet. It is the subject of a note in the Q.M.C.J Vol. 1958/61 page 275.

Hyrax. R.I. 1.80

Also obtained a little of this at the Quekett.

From the type of bottle and the great difficulty I had in removing the stopper I should think it is very old, maybe 50 years. Have tried it once but it was not easy to use, or my handling was wrong. I do not know whether it was right

but I used Benzene for clearing because that had helped me to get the stopper out. I only got one clear mount out of six, the others were ruined by bubbles.
J. Grover of the P.M.S. uses it successfully; better check with him before using again.
Naphrax. R.I. 1.76-1.80
Obtained a small quantity through the Quekett. Have tried it a few times but a bit awkward, sometimes bubbles and can't get rid of them. It comes from Col. Wm. D. Fleming of the U.S.A. See "The Microscope" Vol VI p. 143., also J.R.M.S Vol. 63 p. 34 and Vol. 74 p.42. Solvent Toluene.
Microps 1.63
A proprietary of Flatters & Garnett. In natural resin form. I purchased some but have not used it yet.
Aroclor 4465 R.I. 1.66
Bought some of this from Mr. Darby, also in resin form but have not tried it yet.
J. Grover of P.M.S. has. There is a note in "The Microscope" Vol. 11 p. 68
Coumarone. R. I. 1.63
Purchased a small quantity through Mr. Darby, but have not used it. It is in resin form. Two articles about this resin, by Ed Frison of Antwerp, appeared in "The Microscope" Vol. 9 pp 39 and 63, and Vol. 10 p.207. Although his particular use of it is for Plant Sections he says it can be used equally well for diatoms. A little castor oil should be added or cover-glasses may come off. Also wants a strong cement ring.
(See also 'Diatom Notes' for other mountants.)

MONOBROMONAPTHALENE.

This was advocated, and used, by Caballero. J.D. Möller used it in early years, but·later found it unsatisfactory. He then undid his stock of Test Plates, which were in that medium, and re-mounted them in Styrax.
It can be purchased from Flatters & Garnett, but it is difficult to use. See Caballero's description in my Diary No. 4 p.80.

AROCLOR 1262/4465

Note on a box of 60 slides which Mr. W.F. Barnett had sent to Mr. Hobbs of the Q.M.C. to obtain opinions thereon of one or two members.
This box of slides came to me at an opportune moment, as I was considering making up some Aroclor on Mr. Barnett's formula.
I have had some 4465 for some time and had tried it for selected mounts. It was uncertain in respect of bubbles and also in moving diatoms out of position, so I dropped it. I was then advised to mix 1262 with it, and was given some and was referred to Mr. Barnett's article. I have not made it up yet but will do so and see whether it will be any safer to use for selected mounts. According to Mr. Barnett's article it would not seem so. The slides he has sent are all Strewn and there are some occasional bubbles; this may not be serious in a Strewn mount, but to get a stubborn one in the middle of a Selected mount is not pleasing and would not suit me. However, I will give it a trial.
The ideal comparison is that of slides Nos. 61, 62, and 63, a method which I use for comparing with other mountants. It is, I think, the most reliable·comparison, as long as one endeavours to make sure like is compared with like in the case of the material. In this instance they are Strews of *Surirella gemma*, a most suitable species, and one can see that the resolution of 1262/4465 is superior to the other mountants compared with on the same slide.
The mountant also seemed to me to be suitable for the type of diatoms prevailing in the mounts which Mr. Barnett has sent, with plenty of *Pleurosigmae, Naviculae*, etc, but I doubt whether· it would make any difference with more robust and less transparent forms, as I found with 4465 and also other mixtures based on these resins.
Thanks to Mr. Barnett I will now be spurred on into making the 1262/4465 and testing it on the selected mounts.
Cedric N. Walter,
"Rotherwood",
32, Stanley Avenue,
Beckenhem, Kent.
27th. August 1966

MOUNTANTS.

Aroclor 4465.

This resin is obtainable from
Monsanto Chemicals Ltd.
Victoria House,

Victoria St, S.W.1.

They will supply a Catalogue "The Aroclors". This resin is in solid form.

For use,

1. Diatoms on the slide, or the CG.
2. Put small portion of the resin on the slide just near the diatoms, or the mounting position if on CG.
3. Slide on Hot Plate and resin will melt. Gentle heat.
4. CG on in usual way

The R.I is 1.6655 per "The Microscope" Vol. 11 p.68

Dr. Spence in "The Microscope", as above, does not advocate the use of 4465 alone. It is likely to spring from the Slide if it receives a sharp blow. It should be mixed with 1262.

For use of 4465 and 1262 see:

E. Frison. "The Microscope“ Vol 10 p.206
Dr. Spence. "The Microscope“ Vol 11 p.68
W.F. Barnett. J.Q.M.C. 1958/1961 p.278

STYRAX.
Preparation.

Per R.I. Firth

He said the best Styrax is properly made by oneself, and should be made with Chloroform and not Benzene, and this will give a perfect mount.

He said you want to buy from a chemist the untreated Styrax, just as it comes from the tree. ¼-½ lb, but it must be in its original form.

Then mix the Styrax with Chloroform and get a thin solution and this wants to be filtered through filter paper. It will go through the filter paper if you have the mixture thin enough which will depend upon the amount of Chloroform which has been added.

Next, put the result in an old saucepan, add chloroform again, fill with water, then boil it on a gas ring. When boiling see it does not exceed 100 degrees Centigrade. While it is, boiling, pour off the water, without the Styrax, then fill again and repeat process, including the boiling, four or five times. One wants to get rid of any signs of oil.

At the end of all this one should be left with Styrax looking like golden syrup. Then either put it into an incubator to harden, which may take several days, at end of which it should be stiff and light in colour. If dark, no good throw it away. Then put into flat bottomed Pyrex dishes; just spread thinly over the bottom of the dish, and if it can be consistently exposed to good sunshine for a period of weeks it will materially improve it. No glass or cover over the top, left exposed to the air.

May get covered with flies but does not matter because the Styrax is hard and one can pick them off. He was able to make an excellent mixture in 1955 which was a good Summer for sunshine. The quantity so obtained will last for many years

To get it ready for use, put a little in the bottom of a glass tube, fill the tube with Chloroform so as to make it fluid, then filter it. He then puts a little of the filtered result at the bottom of a dipping bottle. Really as little as possible, and it is important that whenever it is used from the dipping bottle, it is exposed to the air absolutely as little as possible.

When that small quantity is used up, take some more from the tube and treat in same way. But always filter it.

STYRAX.
PREPARATION.

R.I. Firth's Method.

Half a pound of CRUDE STYRAX.

Dissolve some or all in chloroform.

Filter into an old saucepan.

Fill saucepan with cold water from tap.

Bring to the boil and at the boil for twenty minutes.

Whilst the water is more or less at boiling point, pour off as much of the water as possible, leaving the styrax.

Repeat that three or four times until no sign of oil in the water poured off.

After last time and as much water as possible has been drawn off, run the styrax into one or more flat bottomed dishes (Pyrex about foot in diameter)

The layer of styrax in the dishes should be as shallow as possible.

Dish or dishes should be placed in open air where can get as much sunshine as possible in Spring, Summer and Early Autumn. Several hundred hours of sun if possible.

Flies and dust will get into the styrax; does not matter as will be filtered out when sunshine treatment ended.

Styrax should become more and more horny and finally, brittle.
At end of sunshine treatment, styrax should be scraped off the dishes and put into a small jar for use as required.
For use. Only a small quantity at a time in the mountant bottle.
Take a little from the storage jar.
Put in a tube and fill up with chloroform, then filter it twice. Hyduro 808.

LIQUID AMBAR STYRACIFLUA.

In the Gardening Club programme on B.B.C.
Television on 3rd. September 1965, Percy Thrower was going round the Gardening Club Garden pointing out various shrubs which had been put in some time ago and their condition on this date. He came to one shrub and said "Here is Liquid Ambar", whereupon I sat up at once. He did not mention the species, only said it had grown very well and strongly; it looked very healthy. I did not know one could grow it in this Country. After the programme I looked up my book on trees - "The Identification of Trees and Shrubs" by F. K. Makins. This relates to any wild or garden tree or shrub likely to be met with in the British Isles. On page 230 appears the following:
Liquidambar Styraciflua L. Sweet Gum, American Red Gum. May. Deciduous. Young branchlets Corky. Leaves alternate, ovate, 6, palmately 5-7 lobed , toothed, long-stalked, turning brilliant colours in Autumn. Flowers small, unisexual, without petals or sepals, in small terminal heads. The tree·gives a beautiful wood often known as satin walnut. East United States.
Also
L. formosana (Hance) is a Chinese tree with 3-lobed leaves.
Would the latter be the one quoted as *S. Orientalis*?
Van Heurck (1896), page 76 says "Styrax is a natural balsam which exudes from the *Styrax Orientalis* Miller, a native of Asia Minor; On page 77 he says:
“Liquidambar is preferable to Styrax. This balsam, which is obtained from the *Liquidambar Styraciflua* is not met with commercially in Europe, but it can now be purchased from Messrs. Paul Rousseau & Co., 17, Rue Soufflot, Paris, purified and hardened according to our method or in solution (when it is made up either with chloroform or benzene, and is preferable for mounting diatoms) Liquidambar is more easily managed than Styrax and has a little higher index of refraction.”
According to Jaeger’s "Source Book of Biological Names and Terms":
Styrax - see styrac.
Styrac. Gr. styrax, genit. styrakos , ancient name for a tree producing a fragrant gummy resin called storax by Pliny and Vergillius Maro.
Ex. Styrac-aceae; Styrax.

MOUNTANTS.
STYRAX/AROCLOR

On 10th April 1966 I received from P.W. Brimson a tube containing a mountant which he had prepared. In his letter he said it was composed of Bleached Styrax in 4-methyl-penton-2-ol, mixed,with Aroclor 5460 in Toluene; the R.I. being 1.64. It had been filtered through the finest paper, Green’s Hyduro 808.
I proceeded to try it out. I made three mounts of species of which I had several specimens in Styrax, which would enable me to make a comparison. My notes were:-
Coscinodiscus elegans. Compared with 9077, 9078. 9079, 9080 Hakodate, Japan and 9136 Oamaru, all in styrax. At 4mm X 12 mine had a brighter image, Brimson's appeared darker.
Actinoptychus heliopelta. Compared with 9006 False Bay, S.A.; 9005, 9047, 9048 and 9070 Dunkirk U.S.A. Here again mine showed a brighter image and clearer detail.
Arachnoidiscus ehrenbergii. Compared with 9057 and 9073 False Bay, S.A. 9164 and 9165 Oamaru and 9249 Kamischev. This was a particularly fine specimen and superior to any which I have so far as pattern and clarity goes; the only thing was that it was dark, darker than mine.
I felt the foregoing was not a fair comparison. I had taken the above three specimens from a Search Slide of mixed diatoms from many localities I believe, so that the comparison was not a true one to the extent that the diatoms concerned were not from the same gatherings. They were probably from different parts of the world. Another factor is that not only can there be variations in the individual diatom but there can be variation due to the methods of cleaning and the amount of bleaching.

I recollected I had sent Brimson some of my Brigger's mountant, so I compared one or two specimens done in Brigger's and as far as I could tell there did not appear to be any difference. It looked to me as if Brimson had based his formula on that of Brigger.

Brimson.	**Brigger.**
Bleached Styrax	Crude Styrax Liguidambar orientalis
4-methyl-penton-2-ol	Benzol
Aroclor 5460	Aroclor 5460
Toluene	Toluol
	Methyl isobutyl carbinol
	Filtrol (I believe Filtrol is not obtainable over here, but Fullers Earth is used instead.)

So now I had a comparison of Brimson's, Brigger's and also Styrax. My Styrax is purchased ready made from G. Gurr & Sons; it looks quite dark in the·bottle but the mount is clear.

I then set to work and planned and prepared mounts in my own particular comparison method, so that the two, or the three, mountants are on the one slide and can be rapidly compared using the mechanical stage.

When making comparisons one wants to ensure that like is compared with like. One factor is the slide; there can be slight differences in slides and the mounts on each would not be done quite at the same time. The next thing is to see that whatever is used in the preparation is exactly the same, except for the mountant itself.

I made strews. The diatoms in the comparisons on each slide are from the one dip of the pipette, the drops on the three, or the two, cover-glasses, being made from that same dip at the same time, and-heated dry at the same time. The quantity of mountant for the three, or the two, was as closely as I could gauge it, the same quantity, in order to get the same depth of mountant.

A further factor is that by this means the comparisons on the one slide of the mounts can be made immediately while the vision of one is still quite fresh in mind, and one will, therefore, pick up differences more easily. If the diatoms to be compared are on separate slides, time is required·to remove the slide, replace it, and refocus; there has to be a marked difference for observation.

To make the comparisons more complete I worked on three grades of diatoms, viz:

1. The very finely marked ones such as *Navicula cuspidata* which is the predominant form in my mount marked "Mixed Diatoms". I had not got, at the moment available any of the usual tests such as certain *Pleurosigma* and *Surirella Gemma*.

(This should have been entirely a fresh water 'gathering from a Pond near Harlow, Essex, which had been given to me, but somehow some fossil diatoms got in. I think I had put some of the F.W diatoms in a tube in distilled water to clean them from any phenol and the tube must have previously had M.F in it and not been thoroughly cleaned. So l call it "Mixed")

2. Medium size diatoms - Kamischev.

3. Large diatoms - Palos Verdes.

So I ran off duplicate sets for each of the above grades giving

a. Styrax, Brimson, Brigger

b. Brimson. Brigger

Brimson's mountant is easy to use and has one great advantage - it sets hard immediately it is placed on the cold plate on removal from the hot slate. Brigger's takes longer to harden. This quick hardening is a great improvement on Styrax alone which never solidifies completely.

Another point I noticed with Brimson's and Brigger's mountant was that a little smaller quantity can be used than with Styrax, with the result that the depth of mountant is less and the cover-glass closer to the diatoms.

I found at first with both Brimson's and Brigger's mountants they were subject to bubbles after the cover-glass was on, but placed in the Thermostatic Oven at 75°C, even the most stubborn ones were dispersed although it took varying times. This provided a solution to the problem. I adjust the heat on the hot plate so that the temperature of the slide will rise to 75°C after two minutes. I put the slide with the mountant on, and with the cover glass lying on one end of the slide, on the hot plate for three minutes. Then on putting on the coverglass while the slide is still on the hot plate it is rare to get a bubble. Another thing was to let the mountant stand cold when first put on the slide for about a quarter of an hour to settle down. For selected slides I clear the diatoms in Toluene before putting on the mountant.

The only question I have in mind from the foregoing procedure of mounting is whether the temperature used could in any way deteriorate the R.I. of the mountant. I should not think so because, from tests I have made, the temperature of the slide itself does not rise immediately to 75°C when put on the hot plate. Its initial coldness has to be overcome and the rise in temperature is gradual, reaching 60°C at the end of the first minute, then it slows right down only reaching 75°C during the third minute.

I notice that both Brimson and Brigger use Aroclor 5460. In Brigger's formula he uses it 50/50 with Styrax with a resultant R.I. of 1.64, from which I deduce the R.I. of 5460 as being 1.697. But on looking up W.F. Barnett's article

in the Q.M.C.J 1958/61 page 278 he gives the R.I. of 5460 as 1.660 to 1.665., I wonder which is right? My calculation for the R.I. of 5460 was

Styrax 1.583 + Aroclor 5460 1.697 = 3.280
3.280 / 2 = 1.64 - The R.I. quoted by Brigger

As the resins were used in equal parts, then double the R,I and subtract the known Styrax R.I. and the balance would be that of 5460.

So it would seem to me, but I expect I am wrong somewhere. Technical y I know nothing about Refractive Index and am too old now to sit down and try to understand Sines and algebraic equations and formulae which seem so essential to a proper understanding of the subject. But, and I quote from my P.M,S. Notebook on "Operation Diatoms" now on circuit:

"bearing in mind that many of the detailed studies of diatoms were done in the second half of the last century (and how admirable they were) when mounts were in either Canada Balsam or Styrax, it is remarkable what was determined when you check the observations made with mounts prepared in that period. See the works of Smith, Kitton, Grunow and so many others."

[Note: The content of the pages of “Operation Diatom” are reproduced in Appendix A. Unfortunately the slides referred to are no longer with the notebook.]

My limited experience has shown that some differences must be due to the diatom itself. .I have some prepared in Canada Balsam which are superior in brilliance and detail to the same species mounted in Styrax, but they are large robust species. It would not apply to small delicate structures like *Navicula cuspidata*. Hyrax has an R.I of 1.710 but S.H. Meakin did not recommend it for the larger diatoms.

Now for the comparisons shown on the slides that go with this note, Are the differences in R.I. between the different mountants so obvious?

Brimson’s and Brigger's mountants show a little better resolution, compared with Styrax, and mounts where the markings of the diatom structure are very fine, but not so obvious where the diatoms are larger.

The percentages of increase in R.I. are not very large.

	R.I.	**Increase**
Styrax	1.583	
Brimson	1.64	3.8%
Brigger	1.64	3.8%

Looking at those percentages the difference numerically is a slight one and is not enough to throw up any conspicuous difference. But there may be other technical factors in, this R.I. question which I am ignorant of and which may override the mathematical calculation.

I sent Mr. Brimson the following duplicate mounts:-

Triple. Styrax. Brimson. Brigger
9331 Mixed Diatoms. RFW & MF
9333 Kamischev MF
9334 Palos Verdes MF

Double. Brimson and;Brigger
9336 Mixed diatoms
9337 Kamischev
9338 Palos Verdes

Now this R.I. question reminds me I have a very old slide, a wooden slide of diatoms and the wording on it is "*Strigilis elongata*" I would call it to-day *Pleurosigma elongatum*. The slide, when I first bought it was a bit of a mystery but it was fully explained by W. Smith in his "British Diatomaceae" Vol. 1 page xxxii, where he gives full details how a wooden slide like this is made and it is a dry mount because Canada Balsam "obliterates the more delicate markings". The slide has been drilled right through and that depth allows for the use of a high power objective. I found that with 4 mm I got perfect resolution (using under side of slide) superior in every way to that of the same species where a mountant had been used. ·And this wooden mount must be 100 years old!

This caused me to make some dry mounts to see what the effect was and I have noticed in other species that fine markings are more clearly resolved in a dry mount than with any mountant.

So to round off the present exercise, I have prepared and have sent Mr. Brimson, duplicates of the following:-

Double - Dry and Brimson's

9339. Mixed Diatoms
9340 Kamischev
9341 Palos Verdes

In the case of the Mixed Diatoms 9339 taking *Navicula cuspidata*, and one or two of the others which have fine markings, they are resolved much more clearly in the Dry mount than in the mountant. This is not apparent in the same way in 9540, and 9541 because the diatoms are larger, where in the dry state most of the diatoms appear to be dark and a little dirty. This confirms Smith's view. I do not know how long the dry ones will last or whether I ought to have included in each a minute piece of Phenol.
Stephanopyxis Grunowii. In order to show Mr. Brimson how excellent his mountant is, I am presenting him with a multi-mount of this species containing 30 of its forms. This mount was·prepared exactly as I have mentioned above for selected and was cleared in Toluene before putting on the mountant.
I am sending a duplicate of these notes to Mr. Brimson who will, I am sure, have some useful criticisms and corrections to make, which I shall be glad of. (13.6.66)

P.W. Brimson's comments on my Mountant Notes
paged 1-7 (The page numbers must refer to his letter and these comments are reproduced as they contain information relating to the preceding paragraphs)
Page 1. last para. I agree this is important; for true comparison one must have either two diatoms as alike in every respect as can be obtained, or many slides.
Page 2. You are quite correct of course that my formula is based on that of A.L.Brigger; I am sorry I did not make this clear in the letter I sent accompanying the sample, though I did mention that I should be making some in my letter of 31.8.65.
Your method of making comparison slides is good and can establish the useful limits of a mountant. This I think important since I consider one needs a series of different mountants of different R.I.'s for different types of diatoms. Thus a large *Aulacodiscus* or *Coscinodiscus* can actually look better in Canada Balsam or Styrax than in media of higher R.I. which will give too much contrast. One must choose mountant to suit diatom, and for this reason mounts of many diatoms of "varying fineness" and density are always more or less satisfactory.
Page 4.
(i) Styrax/Aroclor can quite safely be heated to the temperatures you mention.
(ii) The R.I. of Aroclor 5460 is certainly 1.660-1.665; this is stated in a table in the Monsanto Chemicals Bulletin of which I have a copy.
I think it is the Styrax side of your calculation which is at fault.' Styrax, as you well know, is a natural product and it's composition is thus inclined to be variable, hence so is it's R.I. You get good examples and poor ones. The figure of 1.583 which is often quoted as the R.I of Styrax refers to the solution. Now when the solvent (which, unless something like a monobromonaphhalene, almost always has a low R.I., is evaporated, the Styrax attains an R.I. in the region of 1.6. In using Styrax/Xylene alone this is usually a very slow process, but as you observed in using Styrax/Aroclor, it sets hard immediately and therefore attains it's maximum R.I. right away.
Page 5. Last para. As implied earlier in this letter the mountant is not intended to replace Styrax or to be used for very coarse or very fine forms, only to possibly supplement Styrax.
Dry Mounts. As Abbe first suggested, the difference between the R.I of the object and that of the mountant is a measure of the visibility of the object. The R.I of diatom Silex is 1.434 and the easiest way therefore of securing a large difference in refractivity is to mount diatoms dry, i.e., in air where the difference is 1.434-1.000 = 0.434. Although this difference is difficult to surpass (i.e. by using a medium of R.I. in excess of 1.87), a medium of an R.I of only 1.0 robs one of the advantage derived from the use of immersion objectives of large N.A. In passing, it is interesting to note that little advantage is gained by mounting in Canada Balsam (R.I. 1.54) over water(R.I. 1.33) as the indices of visibility are almost identical - this is neglecting the fact that one can use immersion objectives with Canada Balsam, the mounts are easier to prepare, etc.
To return to air though, where observation by means of dry lenses only is intended, to mount dry is quite reasonable, although the method is not much used because it is more difficult and time consuming to prepare a dry mount that will last than one in a H.R.I. medium. Test objects mounted dry (and without fixative) have their place for special study, but they should be reserved for this as the reflections and refractions of the light, and the numerous planes and complicated structure of the siliceous valves causes a confused appearance different from the brilliant appearance they present when mounted in a H.R.I. medium which prevents the production of these troublesome optical phenomena. One final advantage of a medium over air is that the former increases the depth of field in satisfactory focus for a given magnification.
Re Wm. Smith's comments - these were written in 1855 (Vol. 1) when C.B. and air were the only known media for diatom mounting.

The ideal mountant is rather like the elusive Philosopher's Stone; one must therefore go for the best compromise. Points I am currently considering include:-

1) Colour. should be pale, tending towards the green end of the spectrum rather than the red.
2) Melting Point - to be low so that delicate solenoid forms will not be damaged.
3) Boiling point - must be high as a large difference between this and Melting Point will tend to avoid the formation of bubbles.
4) Plasticity - must be constant, i.e. it should set to a horny consistency but not become brittle.
5) R.I. - "suitably high"
6) Easy to prepare
7) Easy to handle - no poisonous fumes etc.
8) Inexpensive
9) Consistency such as to flow evenly between the cover and slide
10) Not subject to deterioration
11) R.I. must not have a high rate of change, e.g. with temperature
12) Suitable dispersion
13) No solvent to lower R.I.
14) Stick tenaciously to glass
15) If heating is essential, the colour should not darken.

There is no particular significance in the order of this list - it's just as I thought of them
From P.W.B's letter 24th. June 1966

PLEURAX.

On 3rd July 1966 P.W. Brimson sent me a small tube containing some Pleurax which he had prepared. He did not give the formula and said he would notify me later of the R.I. He gave a warning that the ethanol, which was the solvent, should be completely evaporated, in the case of selected mounts or the Indian Ink on the inside of the cover-glass would be distorted; further not to use an adhesive which contained Gum Tragacanth.

I checked and soon found the truth of his remark about the Indian Ink. The answer was a carmine ring, carmine being a pigment is quite unaffected by anything in the composition of the Pleurax.

Bubbles are quite a problem with this mountant and I tried two ways of using it to try and overcome the bubbles.

(a) The cold method.

This consists of putting a drop of Pleurax on the slide, letting it stand cold for a quarter of an hour or so until all movement has ceased; then while it is cold putting on the cover-glass with the carmine ring. After that put the slide on the hot plate at a temperature which will rise on the slide to about 50°C at the end of three minutes. Observation shows that after about one minute tiny bubbles start to come from the centre in one direction, the number would then increase to a steady stream all hurrying out to the edge. This would be followed by what appeared to be a minor explosion and a sort of wave would go out in the same direction as the bubbles out to the edge. After that everything becomes still by the end of three minutes. This would sometimes result in no bubbles at all when examined or at other times there would be a few very small ones. In the latter case if the slide was placed in the thermostatic oven at 70°c for about an hour the bubbles would disappear.

(b) The hot method.

Here after putting a drop of Pleurax on a slide and letting it stand for a quarter of an hour it was then placed on the hot plate at same temperature to above and remain there for three minutes. Bubble movement would occur after one minute rising in the centre and going out to edge in a lump. This would clear by the end of the third minute. The cover-glass which had been lying on the slide the whole time to attain equal warmth, was then put on while the slide was still on the hot plate. This was, like (a) above, sometimes free of bubbles and sometimes not.

There was, however, one resultant difference between these two methods. In (a) the cold method, the mountant was not hard after completion; it was similar to Styrax, but it became hard if treated like a Canada Balsam slide, by putting it in the oven at 70°C for twenty four hours. The excess mountant could then be quite easily scraped off with a razor blade.

But with the Hot method (b) above the mountant hardens immediately the slide is taken off the hot plate and put on the cold one. If it is clear of bubbles and all else in order, the excess can be scraped off at once.

Another factor with this mountant is the effect on the diatoms. In some cases the evaporation is too violent and moves the diatom right out of its position; in others it causes the diatom to burst open or break. I think the latter is the sudden evaporation of the solvent while inside the diatom.

I did some Strews, comparison mounts between Styrax and Pleurax. They were done using the hot method and where there were any bubbles in the slide, an hour or two in the oven cleared them, although not completely in one or two instances.

I had tried it in some of the more robust diatoms like *Actinoptychus, Arachnoidiscus*, but they were very dark, which was why I only did strews of the freshwater types which are thinner and more transparent.

I shall not use this mountant for a group because of the bubble difficulty, the possible displacement of diatoms or damage to individual diatoms.

Mr. Brimson mentioned "ethanol" as the solvent. In my ignorance I did not know what it was. Later I happened to discover it was Ethyl Alcohol which is 95% of Industrial Methylated Spirits. I tried using the MS to clear the diatom before putting on the Pleurax but I do not think it is quite suitable and better not to do so, trusting to the mountant, under heat, to fully permeate the diatom.

On the 26th July, H.H. Gleave at the Quekett told me he was making some Pleurax and had the formula. He was good enough to send me a photo-copy of the article in the R.M.S. Journal of 1949 by G.D. Hanna in which full details were given. It was a pity I had not known of this earlier because a fortnight previously Hanna had come to the Club and I had been introduced to him. In the course of our talk I asked him whether he used one or several mountants. He told me, several, depending on the type of diatom, but for the very highest resolution he used Hyrax. He never mentioned Pleurax. Had I only known he had introduced that as a mountant I should certainly have questioned him about its best use, He was over from California for the R.M.S. Centenary.

The R.I according to Hanna is about 1.75 I notice.

(P.W.B. in his letter 12/8/66 says his is 1.635)

My Mountants and the effect of Time.

Time seems to have affected such diatom mountants as I have, in different ways, even with the same medium.

Styrax.

George Gurrs.

This goes very dark but retains its liquidity. It shows a thick band of sediment at the bottom of the bottle (glass bottle with screw-on cap).

R.I.Firth.

When he fixed the mechanical finger on my microscope, three years ago, I obtained a little of his own styrax, which he had prepared in 1955. It was in a glass tube with cork stopper and was light in colour and quite liquid when I received it.

I have not used it but to-day it looks just a dark solid mass at the bottom of the bottle. His solvent is chloroform; presumably the addition of Chloroform will liquify it and render it convenient for mounting.

Thum, 1911.

I obtained this from a deceased member's Estate at the Q.M.C.·about two years ago. It is in a small ground glass stoppered bottle, and is labelled "Styrax in Benzene. Thum 1911". If the date on the label is correct it is 55 Years old, but it has retained its clarity and liquidity, and no sediment. I have not used it yet.

Naphrax.

Obtained this two years ago. In a glass bottle with a metal cap. Now solid, but retained its clarity.

No sediment. Solvent is Toluene and presumably will require its addition to be ready for use.

Hyrax.

From a deceased Estate at the Q.M.C. two years ago. In a small glass stoppered bottle. Liquid, clean and no sediment.

Styrax/Aroclor.

Brigger's.

In small glass bottle with metal screw on cap. Very clear, and very liquid. No sediment.

I have had it two years.

Brimson's.

In glass tube with cork stopper. Same as when I received it last April. It was not so liquid as Brigger's.

Pleurax

Received this beginning of July, prepared by P.W. Brimson. In glass tube with cork stopper. Is getting stiffer and darker.

Aroclor 1262

Received this from. P.W.B beginning of July when it was quite liquid. Today it is solid, but glass clear. Presumably heat will dissolve it. In a glass tube with a plastic screw on cap. (See E. Frison "The Microscope" Vol.10. p.206.

Aroclor 4465

Received this about four years ago; it is in a tin tube with tin screw on cap. When I first received it it was in so many pieces of resin; to-day it looks as if it had been heated and melted into one mass at the bottom of the tube. This is a puzzle, in view of its high melting point, and has not been near any heat, having been at the back of a drawer in my Study, Appropriate heat will no doubt liquify it again.

I mention the container of each medium in case that or its cap in any way affects the keeping of the medium, such as complete exclusion of air.

NAPHRAX.

Full details of the process of preparing this Mountant are given in J.R.M.S· 1943/44, page 34, by William D. Fleming, of U.S.A.
A shortened version of the process was given by Dr. Spence in "The Microscope" Vol.6. p.143.
A most long and complicated process requiring a laboratory to carry it out. It is a synthetic resin, the basis of which is Napthalene, Formalin, Glacial Acetic Acid and Sulphuric Acid.
From observations by Col. Fleming in the J.R.M.S. above "mounts made with the resin do not harden as quickly as those with Balsam", and for diatoms, "the hardening may be conveniently hastened by placing the slide on a hot plate heated up to a temperature short of causing bubbles , to form under the cover glass".
In another place he mentions:
After final filtering "the solvent is then evaporated from the resin at a temperature not exceeding 100°C".
In a letter from Col. Fleming, published in "The Microscope" Vol. 6, page 222, he said:
"Since my return from war duties I have developed a process for purifying the crude resin which I feel greatly improves it by removing the by-products which at times have caused precipitates in the finished mount. As soon as patent procedures go through on this, I shall publish details of or this purification process. In the meantime, I·believe the original process mentioned by Spence will give a usable product provided the resin is dissolved to a very high dilution, allowed to stand and any precipitate filtered off. The resin in dilution suitable for mounting has a high viscosity, and if allowed to dry under the coverglass in the cold is apt to develop lacunae. For this reason it is necessary to heat the slides to 90-100°C in order to melt the thickened resin and so lower the viscosity as to permit it to flow freely".

Classification of Slides.

Card Index, divided into four sections: -

1. Selected.
2. Type
3. Geographic
4. Sundries.

1. Selected.
In alphabetical order by Genus, with one card for each species. A separate card for the unidentified species of each genus, i.e. *Biddulphia* sp. The first card in each genus is for Genus Slides.
The information given on the species slide is:

- Slide Number.
- Locality
- Number of diatoms where more than one.
- Diary reference.
- Mountant
- Degree of perfection.
  - X = 3rd class
  - XX = 2ndpclass
  - XXX = 1st Class
  - XXX = Superb.
- 2nd and 3rd class are only there until there is a better one. Can happen with scarce diatoms.
- Source of identification
- Measurements.

On the back of the card any observations relating to particular slides.
The Genus slide card gives similar information and on the back of each card the diatoms are indicated in position in rows or columns and named where possible.
Bought slides are also given on the selected cards, in red.
There is also a card "Unknown" for any mounts the genus of which has not been located.

2. Type
Indexed by locality and give:

Number of Slide
Number of species
Diary reference.
Mountant
Degree of perfection
Tube number - Tubes of diatoms cleaned by different people, for one locality can have differences.

On the back of each card the diatoms are listed by their position in each column or row, giving genus and species where identified.

3. Geographic

A card for each Country on which appears a list of all the localities in that Country, then a card for each of those localities. The slides are all strews and information given is:

Number of Slide
Habitat - RFW. FFW. RM. FM.
Diary reference.
Mountant
Tube number
Cleaned by
Map reference in Lat. and Long.

On the back of each locality card appears a list of all the selected and type slides relating to that locality cross referenced to the other sections.

4. Sundries.

This is for slides which do not come under the other three headings, such as:

Comparison Slides re mountants, etc.
Fragments, by localities
Groups of Diatoms, locality unknown.
All bought. Identification, where known, given on back of card.

ELECTRIC LIGHT BULBS.

Use Opal Bulb. The name is stamped on the centre of the bulb. This can be removed by using damp cloth dipped in Ajax and hard rubbing.

THERMOSTATIC OVEN.

Obtained from Gerrard & Co. Ltd, their Economy Oven, £15. 15. 0d. Calibrated for any temperature from 50-200 degrees Centigrade.
Very satisfactory. Will hold about 80 slides.

PROJECTOR.

I have a Watson Prism Eyepiece Projector. It is only suitable for low power subjects, projected on to a screen, matt white paper, at about 8" away, and picture is small. It fades considerably with any enlargement.
So far, I have not found it of any use for drawing Diatoms. They are very small and require high power; here again the picture fades if the power is raised. Very high illumination would be required to overcome this and will not work with an ordinary microscope and lamp.
It would be possible to get a picture of sorts, Camera Lucida fashion, by setting the microscope horizontally so that the lens would project downwards on to a piece of paper, and the Regulite set so that it is illuminating right up the tube of the microscope. To get anything but the tiniest picture it would be necessary to build up the microscope and the lamp so that the distance from the prism to the paper was nearer 8", but I do not think it would be of any particular value as the highest objective usable would be 16mm.
For general use of the Projector see my Methods book.

MEASUREMENT.

1. Micro Graticule in X10 Eyepiece.
2. Stage micrometer on stage, and focus it.
3. Extend Drawtube to 17.3 to synchronise the 10s on the Stage Micrometer with those in the eyepiece.

Measurement of the eyepiece on the Stage Micrometer will vary with objective. Following table should be correct for Service I

Draw Tube	Objective	Eyepiece
17.3	16mm	10 = .013, or 13µ per section of eyepiece
17.3	8mm	10 = .006, or 6µ per section of eyepiece
17.3	4mm	10 = .003, or 3µ per section of eyepiece

Points to watch when using above table to measure an object are:

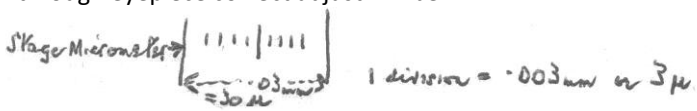
a. Set of Drawtube
b. Watch objective and eyepiece combination

Measurement is easier with higher magnification, and the one I use is 4mm X10.

The above applies to both the Service and the Kima Microscopes.

Van Heurck gives measurements in c.d.m.
C.d.m. = hundredths of a millimetre, $^{1}/_{2500}$ of an inch, or .01mm = 10µ

With Drawtube 17.3, then through eyepiece correct adjust will be



Approximately three divisions of the above would = 10µ, or 1 c.d.m.

COUNTING DOTS.

Per Carpenter 8th Edn. p. 1117.
In a photomicrograph of a diatom amplified 735 diameters 12 dots can be counted in .3 of an inch.
At what rate per inch is the structure in the diatom?

(1) $\frac{\text{Magnifying power x number counted}}{\text{space counted.}}$

$$\frac{735 \times 12}{.3 \text{ inch}} = 29{,}400 \text{ per inch}$$

(2) If the answer is required in the rate per mm., the space in which the number is counted being in inches as before, then, because 1 inch equals 25.4 mm

$$\frac{735 \times 12}{.3 \text{ inch} \times 25.4} = 1157.5 \text{ per mm}$$

(3) Suppose a rule divided in mm. is used to determine the space in which the number on the photomicrograph is counted, and the rate per inch is required; if 12 dots can be counted in 7 mm, then, because 1 inch: 25.4 mm

$$\frac{735 \times 12 \times 25.4}{7\text{mm}} = 32{,}004 \text{ per inch}$$

FINDER.

Very useful for recording the position of a diatom in a Strew. Very small ones are easier found this way than by taking the vernier readings

I have two, one I purchased from Scott at the Q.M.C. and to avoid confusion I call it Scott. The other is a New England Finder sold by Graticules Ltd. It is differently marked and I do not use it. It is a reserve in case the first one was lost or broken.

I have two Service Microscopes, Service I in main use, and Service II identical with the other but has the Mechanical Finger fitted.

The Vernier readings are not identical, although the mechanical stages are meant to be the same.

The variation is as follows:-

Service I	Service II
91¾ - 28 $^{5}/_{6}$	91 - 28

The correction required if have say a Service I reading and want to use it for Service II would be to deduct ¾ from the horizontal and $^{5}/_{6}$ from the vertical.

EYEPIECE GRATICULES.

I use X12-in eyepiece for mounting.

The horizontal line should be aligned to the edge of the slide when put on microscope, before any mounting, and constantly checked.

Suppliers of Graticules of all kinds:-

Graticules Ltd.
Bath House,
57/60 Holborn Viaduct. E.C.1.

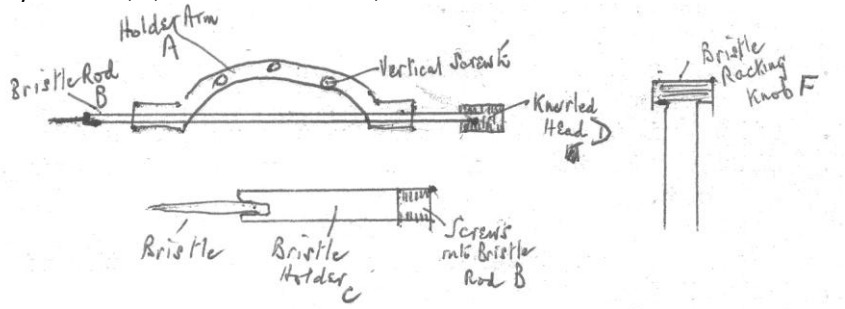
I also have one in lined squares, covering the whole field of view, every tenth line accentuated.

Use X10 or X12 eyepieces.

Using the one shown above one can get diagonals after one has got some diatoms in position horizontal and vertical, by turning the lines into the diagonal position required, but keep checking the horizontal.

MECHANICAL FINGER.

Supplied and fitted by R.I. Firth, 6, Windover Crescent, Lewes. Sussex.



<u>To set Finger.</u>

1. Holder Arm A is off microscope.
2. Set up microscope with Regulite.
3. With Bristle Holder C unscrewed, push the Bristle Rod B through Holder Arm A.
4. Leave room for the Rod B to be pushed further through from Knurled Head D end.
5. Check Rod B revolves easily by turning Head D.
6. Fit Holder Arm A on to the microscope.
7. Check angle to clear objective and tighten vertical screw E with spanner.
8. Rack up objective right out of the way.
9. See Bristle Knob F is racked up.
10. Loosen Lateral Screw (underneath) and swing Holder Arm A out 45 degrees. Tighten.
11. Loosen Vertical Screw E, raise forward end of Holder Arm. Tighten WITH GREAT CARE.
12. Screw Bristle Holder C into the Rod B.
13. VERY GENTLY. Just slightly loosen Vertical Nut, swing arm down, then loosen lateral nut to bring back to operating position, Watching Bristle all the time.

14a. Put a Search Slide on and get objective focussed.

14b. Remove Search and put on a plain slide.

15. See objective is well racked up.
16. Turn Regulite on.

17. WATCHING FROM SIDE. Open S.S. Diaphragm and partly close lamp diaphragm. See tiny circle of light on slide.
18. Rack down Bristle Knob F about 9 turns. Leave one or two turns.
    VERY GENTLY & VIEWING FROM SIDE
19. Slightly loosen vertical screw E enough to permit slight movement of Holder Arm A.
20. Tilt Bristle down until just touching slide.
21. Should see its shadow coming up and two points meet.
    SEE BRISTLE DOES NOT BEND.

21a Rack S.S. Condenser right down and Bristle visible.

22. GENTLY BUT FIRMLY -
    Hold Arm A in position with left hand.
    Gently make finger tight with right hand.
    Check position with S.S. Condenser down.
        NOW LATERAL
24. Loosen Lateral Nut very slightly
25. Viewing from side, see tip Bristle in centre of spot of light.
26. Tighten gently by hand, then by spanner -
27. RACK UP BRISTLE F
28. Remove plain slide

29 Replace with a Search Slide

30a. Adjust Regulite and sub-stage.
30b. Focus microscope on diatoms, one in a blank space.
31. Rack down Bristle F, noting number of turns for exact focus.
32. Check Bristle for
    a. Centre position
    b. Picking up
    c. Revolving.

REMOVE SEARCH SLIDE when making adjustments to Bristle and replace with plain slide.

WHEN TESTING BRISTLE FOR USE

Remove plain slide and replace with Search slide.

To CENTRALISE BRISTLE

Rack up Bristle F and focus it.
Gently turn and <u>pull</u> knurled Head D

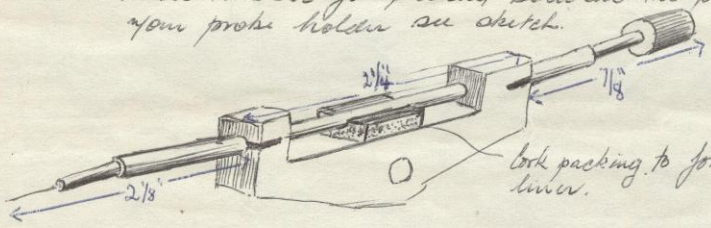
Rack up Bristle F and focus it.
Gently turn and <u>push</u> knurled Head D.

Dear Mr. Walter.

In this parcel you will find:-

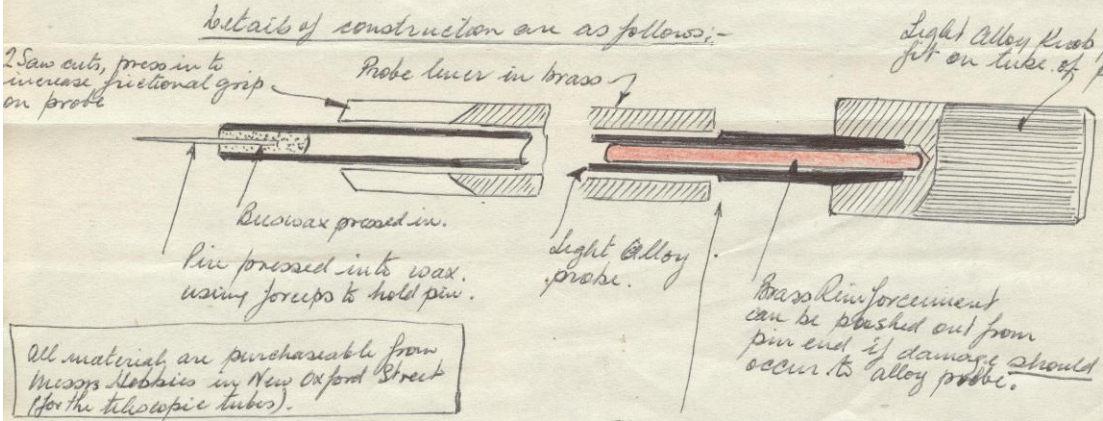
(a) The micro probe.

(b) Some pieces of cork which I think will be better than indiarubber for packing between the probe liner and your probe holder see sketch. (Rubber contains an element of sulphur which may cause corrosion!



(c) A small piece of beeswax. in the plastic box.

Details of construction are as follows:-



All material are purchasable from Messrs Hobbies in New Oxford Street (for the telescopic tubes).

THIS IS THE VULNERABLE POINT SHOULD THE KNOB BE KNOCKED.

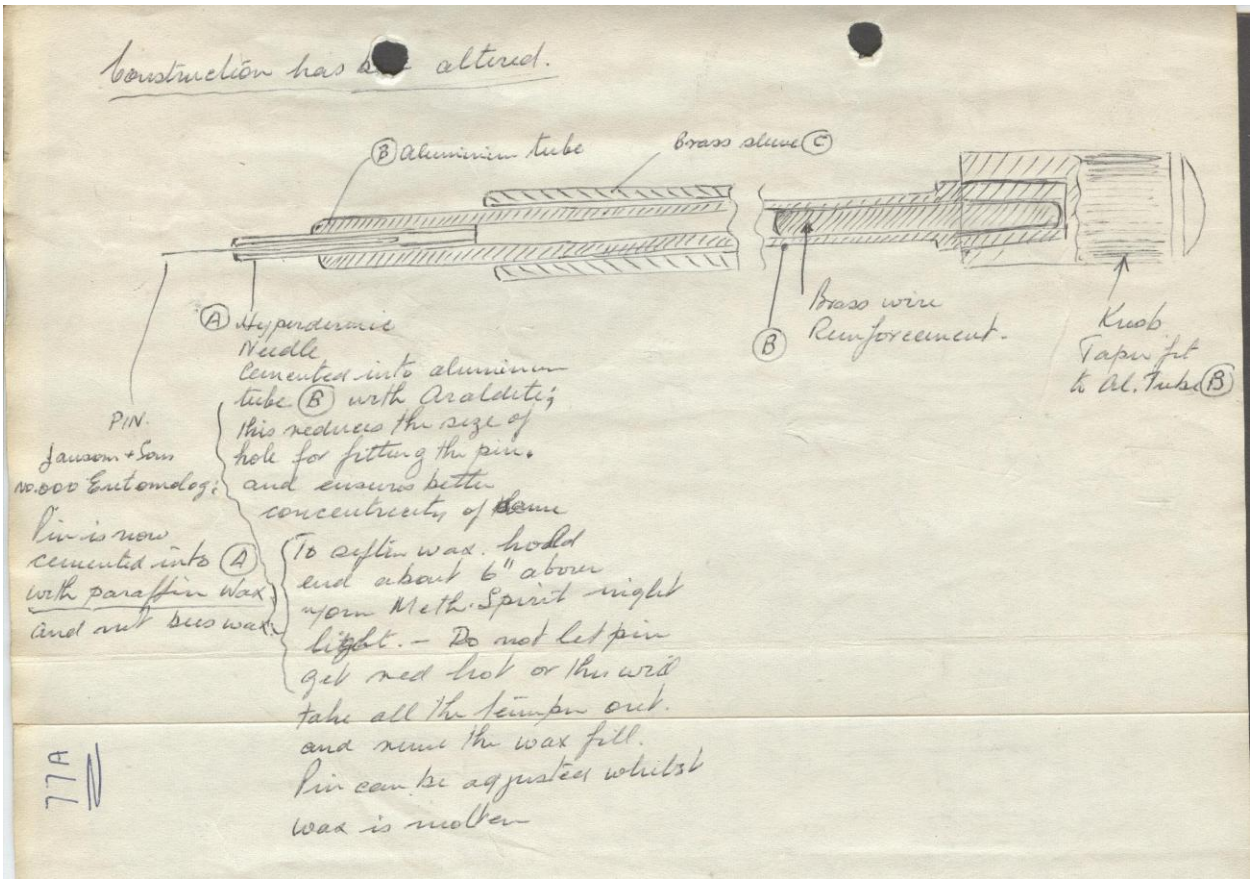
P.T.O.

To assemble:-

(1) Withdraw probe from brass liner.
(2) Enter liner into the probe holder of your micro Manipulator
(3) Cut cork with sharp scalpel and make wedge
(4) Insert probe and adjust for your working position of point under microscope.

Hoping you will find it will will ease your job!

Eric Tripp
28/7/66.



OBJECT MARKER.

I purchased one from Flatters & Garnett; it has an extension piece which screws into the nose piece and the marker portion screws into the extension.
Put the marker on nosepiece, so that it follows the 16 mm.
Find the object with the 16 mm objective
Rack up nosepiece after turning the Marker into the place of the 16 mm.
Wet the rubber tip of the marker with saliva.
Draw a freshly inked pad across the tip of the marker
Rack the marker down to touch the slide
It should leave a small round circle.
Take off slide, turn it over, put it on the ringing table, centring the ring just made by the marker.
With Gurr's Glass Ink put a ring on coincident with the marker ring.
The marker ring can now be rubbed off. The Gurr's ring is not easily removable and in any case is under the slide and will be fairly permanent.

Method given by Stephanides:
Set Microscope so that Stage is horizontal.
Focus object 16 mm and place piece of Oil Immersion paper under the slide.
With Sub Stage Diaphragm, close it until only very tiny disc of light visible.
With Indian Ink make three dots, triangular formation level with edges of the disc.
Object will be in the triangle.
I have not tried this, but if I did so, I should turn slide over and mark the dots with Gurr's Glass Ink, for reasons given above.
Useful for marking a diatom in a spread or strew.

REFRACTIVE INDICES.

Mountant	R.I.	Index of Visibility
Monobromonapthaline	1.658 (Carpenter p.521)	
Silex of Diatoms	1.434	
Canada Balsam	1.526	9
Styrax	1.583	15
Styrax/Aroclor. Brimson.	1.64	21
Styrax/Aroclor. Brigger	1.64	21
Clearax	1.666	25
Hyrax	1.710	28
Naphrax	1.76	33
Glycerin	1.473	
Albumen	1.350	
Gum Arabic	1.512	
Dammar	1.520	
Sirax	1.80 (Johnson)	
Realgar	2.549	
Toluene	1.50 (Gray)	
Xylene	1.50 (Gray)	
Benzene	1.50 (Gray)	
Chloroform	1.45 (Gray)	
Castor Oil	1.49 (Johnson)	
Pleurax Brimson.	1.635	

Index of visibility for obscure markings is the difference between the R.I of the Diatom Silex and the mountant, multiplied by 100

KOHLER ILLUMINATION.

PRELIMINARY.

The Microscope.

X6 Eyepiece.
Drawtube 160
16 mm Obj.
S.S. Condenser right up
S.S. Condenser Iris fully open
Filter carrier in right position.
Plano Mirror
See all lenses perfectly clean.

The Regulite

See lamp holder right in
Face wall:
See filament is vertical. Move lamp housing to adjust.
See filament in centre of lamp disc. Use centralising screws.
See all lenses are perfectly clean.

To Set-Up.

1. Distance lamp source (i.e. electric bulb) from object, Lamp bulb reaches to W of Watson on lamp housing.
5 inches from toe of microscope to front edge of transformer = six inches from W to mirror, plus 4 inches mirror to object = 10 inches.
2. Lamp lens right in and Iris open
3. Focus lamp on mirror
4. Focus lamp in microscope and adjust lamp to show filament in centre of field of view.
5. Close Sub stage Iris and partly close lamp iris. Look round microscope and the filament should show as a small circle in centre of the closed Sub Stage iris.
6. Open sub stage iris again.

7. Put slide on Micro. Focus it. Do not alter focus after
8. Remove slide
9. Rack sub stage condenser right up. And close sub stage iris.
10. Rack Sub Stage condenser down until it shows circle of Sub Stage iris with either a complete and even blue or orange rim.
11. Centre this circle using Sub Stage centralisers.
12. Open the iris to just fill the field of view.
13. Close lamp iris and rack up the Sub Stage condenser. If the light is not central move mirror and/or lamp to adjust.
14 Open lamp iris to just fill field of view.
15. Check with slide and slightly lower Sub Stage condenser to focus it and give evenly clear bright light.
16. See filament shows central. While slide is on and focussed put lamp lens right in

Filament will show quite clearly but below the object. By moving lamp slightly get the object into centre of filament. Move the lamp lens rod to the left and see the filament rise up to the object. With this rod at 11 o'clock the filament will completely fill the object.

7-16 should be repeated on each change of objective.
If 16 mm objective is to be used, remove the top lens of the Sub Stage condenser.

DRAW TUBE CORRECTION.

Correcting for Spherical Aberration by means of Draw Tube.

Stephanides page 88.
Oliver. Page 110.

Per Stephanides.

Bring tiny particle to centre field and focussed.
Fine adjustment raise and lower
If correct the image should be same at lower or higher,
If not
Move fine adjustment up or down and see which way produces a sharp ring, and other side misty.
If down for ring, raise up, and if up for ring, lower tube.
Now to focus the particle. Not to use fine adjustment. Lengthen or shorten drawtube.
Now test with fine adjustment and continue doing above until get same appearance each side of focus, so Test with fine adjustment, Correct with draw tube.
On change in eye piece adjustment should be made by draw tube and NOT fine adjustment.
From low to high, lower tube
From high to low raise tube
This wants checking on every mount for 4 mm or higher.

ACHROMATS AND APOCHROMATS.
COMPARISON OF ACHROMATS & APOCHROMATS

Watson	**Ach**	**Apo**	**Ach**	**Apo**	**Ach**	**Apo**	**Ach**	**Apo**
Focal Length	16mm	16mm	8mm	8mm	4mm	4mm	2mm	2mm
Primary Mag.	X10	X8	X20	X20	X40	X38	X100	X90
N.A.	0.28	0.30	0.50	0.65	0.70	0.85	1.30	1.37
Free Working Distance	7mm	7mm	1.5mm	0.56mm	1.0mm	0.18mm	0.12mm	0.12mm

REMOVE GREEN FILTER.

Koritska. Milan
Focal length 3mm. Oil Imm.
N.A. 1.40

Visibility and resolution of Microscopical Detail.

This was the title of a series of articles by C. van Duijn, Jnr., published in "The Microscope" as follows:-
Vol. 11. pp. 196, 222, 254, 273, 301.
Vol. 12. pp 16, 38, 92, 131, 185, 201, 244 269, 298.

It is not my intention to attempt to summarise his series, but merely to pick out from them any references he makes to Diatoms.
In Vol. 11, page 200, is shown a photograph giving details of *Pleurosigma balticum*, in Styrax, at three magnifications,

a. X 1250
b. X 2,500
c. X 4,000

These magnifications must be some combination of the camera and the microscope. This is followed by an explanation of how these photomicrographs can be used as a test for eyesight in the calculation of the visual acuity, from which the observer's lowest useful magnification may be calculated.
I quote the following from page 225, Vol. 11
"Dealing with a point source for illuminating the specimen permits considerable simplification in mathematical treatment of microscope image formation and in fact it is the only condition that has been mastered more or less quantitatively. In practice, however, light sources are in general use which do not bear any resemblance to an ideal point source The practical effect of using an extended source instead of a point source is an increase of resolving power, but coupled with decreased contrast. These effects can be demonstrated easily by examining a diatom with very delicate structure (such as a *Pleurosigma*) with a completely diffused light source and then, using the same optical system, exchanging the diffused light source for a point source. Stopping down the condenser in both cases until diffraction fringes appear, it will be found that this spurious effect occurs quicker with the point source than with the diffused light source. On the other hand, if the condenser diaphragm is opened wide enough to avoid diffraction fringes and to allow complete resolution contrast will be better in the image using the point source. On repeating the experiment with an ordinary Abbe condenser and comparing it with a fully corrected condenser of the same aperture, it will be found that in this respect no significant difference in performance between the two condensers can be observed with the diffused light source, whereas with the point source the corrected condenser gives better results. However, the maximum condenser aperture which can be employed without producing flare will be higher with the corrected condenser in any of the experimental conditions.

On page 98, Vol. 12, I quote:-
"In ordinary achromatic objectives pairs of colours are brought to one focus, viz. violet with red, blue with orange, and blue-green with yellow, only the blue with orange pair being complementary and yielding white. Consequently, small colourless detail, such as the dots of a diatom scale, may appear with spurious colours and these colours may be used as a test for the quality of the colour correction of a microscope objective. The light source has to be of very high specific intensity and a high power eyepiece should be used to eliminate interference by the ‘colour eliminating mechanism' of the eye, mentioned previously. In the average microscope objective the colours of the secondary spectrum are apple-green and purple but other combinations may occur in some systems, such as orange and blue, or even red and blue-green. With an objective of the first type of correction, the diatom *Pleurosigma angulatum* will show (with normal focus) in mid-rib apple-green flanked by violet lines, while the dots appear purple against an apple-green background.

Vol. 12. page 188
"Contrast of the diffraction fringes increases with increasing difference between the refractive indices of the object's structure and its surrounds. As a practical example may be mentioned the occurrence of visible diffraction fringes at the edges of diatoms mounted in media of high refractive index, such as Hyrax, or Styrax, even if observed with full cone illumination, whereas no fringes may be observable with similar specimens mounted in Styrax at some lower condenser aperture." On page 189 are two photographs of "Diatom frustule"(looks to me like *Navicula cuspidata*) mounted in Sirax, R.I. 1.66 and showing diffraction fringes at two different magnifications, viz. by 650 and by 2,000.

Vol. 12. Page 195
Plate three of *Rhabdonema* under three different forms of illumination. These are related to the following observation given on page 191

"Instead of obtaining a spurious single resolution, by surpassing the 1,000 N.A. limit the increased magnification in reality reveals that he observations made at the N.A. limit are completely spurious. Some of the accompanying photomicrographs clearly show the effects. Plate III."

Vol. 12. Page 202.
A plate of three different photographs of diatoms from Samoa (? *N. phoenicentron*) mounted in Sirax, under three different forms of illumination at different N.As. This is related to the remarks on page 193
"If the illuminating cone is reduced further to N.A. 0.14, a real dark field image is obtained. At further reduction of the N.A. of the illuminating cone conformity of the image detail with the real object detail is gradually destroyed. (Compare the photomicrographs of the diatom *Rhabdonema*). With diatoms containing very fine structure resolution of dots disappears completely as soon as the condenser iris is closed.

Although the foregoing only relates to specific references by the author to diatoms, there is a very great deal in his Treatise which should be paid attention to in the microscopical examination of diatoms. Unfortunately for me, so many of the explanations are taken up with mathematical formulae and data. Fifty years ago I could have followed it all but at my present age it is too tiring to try and turn all that up just for the sake of understanding it.
On page 298, Vol. 12 he gives a summary of his conclusions and also at the end of it a complete index to all the different parts of the subject which he has dealt with. This is very useful for turning up any particular aspect. He says in conclusion "it is hoped that this treatise may have some real value for those microscopists who wish to practice <u>really critical</u> microscopy". This is very true.

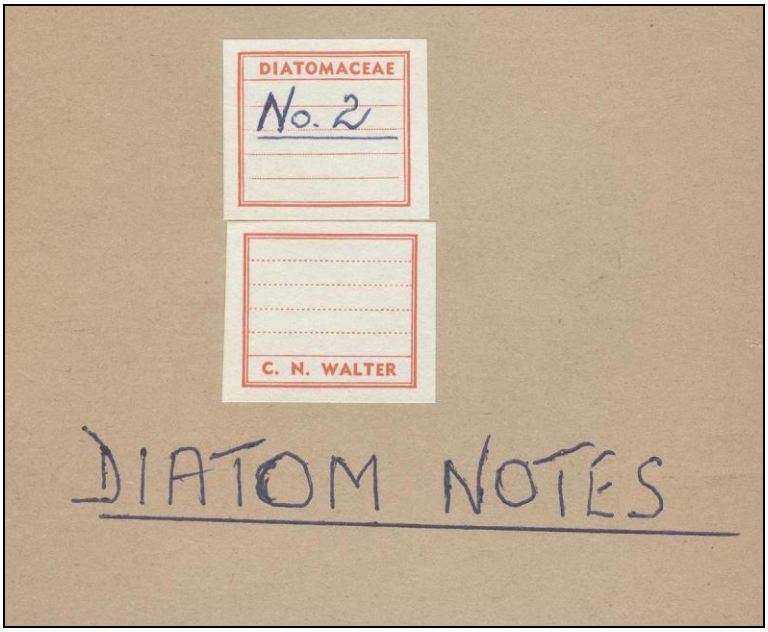
SIEVES.

<u>For use in cleaning diatoms.</u>
I purchased mine from
Endecotts (Filters) Ltd.
Lombard Road,
S.W. 19.
and have the following set made of brass:-
They are 2" diameter and nickel mesh.

Meshes to inch.	Diam. Mesh.
50	500µ = .50 mm
100	250µ = .25 mm
150	160µ = .16 mm
200	125µ = .12 mm
300	80µ = .08 mm
400	60µ = .06 mm

<u>To use them.</u>
Before sifting, material should be given one or two rinses, by letting settle and then decanting the clear liquid.
Use evaporating dish with water in it.
Hold sieve in left hand with mesh just immersed in the water.
Small quantity of turbid fluid poured into sieve. Very small quantity.
Move sieve slightly up and down and gently tap right hand side with forefinger.
Fine material will go through mesh, leaving larger diatoms and particles of earth in sieve. Mud etc, will also get broken up and pass through.
With wash bottle pour a little into the sieve and repeat above procedure. Do this once or twice.
The turbid liquid which has passed through should be retained and examined.

Use of Residue in mesh:-
Invert over an evaporating bowl.
Pour water from wash bottle on to underside of mesh, also use pipette on the inside to clear.
Pour into flask for preservation.
Rinse material in basin with wash bottle.
Should now have a collection of nearly free diatoms, i.e. clear of debris.
Do this through meshes of various sizes.



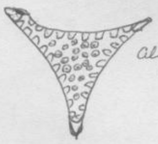
Diatomaceous Ooze.
Note per W. Wilson. P.M.S. No. 346
"Diatoms, being plants, form the basic food of many animals and obviously had to precede them in world evolution. They feed sponges, oysters, small crustaceans which, in turn, become food for fishes and even large animals like whales. Where they flourish so do fish.
They live at the surface of the sea and are kept afloat by their minute size (1/5000" to 1/30" and shape of their delicate skeletons or cases made of silica. Flotation is helped by presence in some of long spines, extreme elongation, even inflation of cell, chain connection, circular arrangement of several, formation of large area such as circle etc. Also increased friction with water helps, provided by the surface sculpturing in bands, lines, dots, etc.
When dead the cases begin to fall and must take years to reach the bed of the ocean (if ever) where depths can reach 5550 fathoms, that is, over six and a quarter miles. Below 2700 fathoms however silica is dissolved and below that only red clay is deposited, formed by the prolonged action of sea water in volcanic dust - all that has survived the journey to the bottom.
Where the depth is suitable inorganic material and diatoms are laid down to form a mud or ooze and in course of time grow to great thicknesses. A Slide l shows a sample of un-cleaned matter consisting mainly of diatoms of various kinds and a very small number of foraminifera. The diatoms are mostly circular showing that this is the form of greatest buoyancy and therefore capable of reaching the sea bed oftenest.
Diatoms, of course, are microscopic unicellular plants containing chlorophyll and a brown pigment. The cell wall is impregnated with silica comprised of two halves (hence the name) one of which overlaps the other.

Red Sea Ooze.
The almost complete absence of diatoms is puzzling but might be explained by the fact that they thrive best in cold waters especially around the poles, particularly in the Antarctic. The largest area of diatomaceous ooze occurs in both sides of latitude 60° S - that is in Antarctic regions, extending all round the world. There is another band in the North Pacific Ocean along the line of latitude approximately 50° N.

1

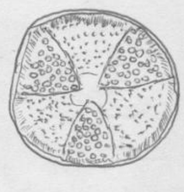
Per R.E. Platten Slo.

Trinacria pileolus



Actinoptychus halionyx.
from Sharks Tooth Hill – Calif.
This is very like splendens main difference
hyaline centre in splendens is star shaped long points
on focusing down. In halionyx it is
more or less central.

Actinoptychus densistosus



Per R.E. Platten.
See Le Diatomiste Vol 2 Plate V.
p 82 my copy

Sceptroneis caduceus
Sharks Hill. Caly

3

MORENO SHALE. CALIFORNIA
CRETACEOUS. FOSSIL
Per R.E. Platten.

Actinoptychus rotula
design very blurred.

Gloriptychus callidus — Lept VII

Aulacodiscus Archangelskianus — 9049

Coscinodiscus steinyi

" obscurus. — 9044/5

" subtilis — Lept VI

Aulacodiscus sagittarius

Stephanopyxis appendiculata

Triceratium dignum

Hemiaulus polymorphus

Trinacria insignis

" mucronata — 9034/5

Stephanopyxis Grunowii

Melosira

Liradiscus

Trinacria aries

4

Moreno Shale (Cont)

Biddulphia

Melosira fausta

Kentrodiscus Blandus

Coscinodiscus oblongus

Platten took his information rather from a
paper by John A Long, Dingley P. Fuge, and
James Smith in Journal of Paleontology Vol 20
March 1946. Nov.

5

Barbados
Per R.E. Platten.

Triceratium glandarum

Coscinodiscus elegans
Supp 13. Does not appear
Do not appear hexagonal

Gos 19/15. — T. Venulosum

9064 — Biddulphia rigida

Gos 2/324 — T. Crenulatum

Gos 1899 — Kittonia Elaborata

Gos 3/4 — T. rugosum

" 6/18-19 — Hemiaulus ornithocephalus

" 11/58 — Aulacodiscus janischii

mine — Stephanopyxis Grunowii

6

Bacon's
Per Platten

9064 Melosira Oamaruensis

9064 Triceratium Novam Zealandicum
Compare with T. Dobreeanum

9064 Actinoptychus Vulgaris

6/16 G.S. Triceratium aculis cordes

9064 Melosira clavigera
Lapart. Reference Pl 4

5/9 G.S. Coscinodiscus spiniferus

Triceratium

Taken from a slide (?) mounted by Oamaru per R.E. Platten

Kinkerianum

G.S. 19/14

Pseudonervatum

G.S. 19/19

dots radiating from centre but in straight lines to edge

Dobreeanum
Var Novae-Zealandiae

favus quadrata

Crenulatum

G.S. 2/3.4

8

Triceratium (Contd) Oamaru

Coscinoides

G.S. 19/13

Very small
Dots like Coscinodiscus

½ size of Crenulatum

fractum

Very wide heads

Castelliforum

½ size of fractum
& corners are narrower

favus

Easy to identify

favus fimbriatum

Junction hexagons like favus

glandarium

undatum

radiating large dots from centre

9

Triceratium (Contd) Oamaru

Undulosum

G.S. 19/15.16

A faint pattern in centre & a few dots on it
The borders look like processes
The three corners hyaline centre then tiny dots

Trinacria ventricosa

G.S. 5/1.2.3

hyaline centre & corners.

Triceratium undatum
(per R.E. Platten)

Note the glandarium shape in the centre
Outline is not so sharply crenulated as the c

Triceratium capitatum
(per R.E. Platten)

10

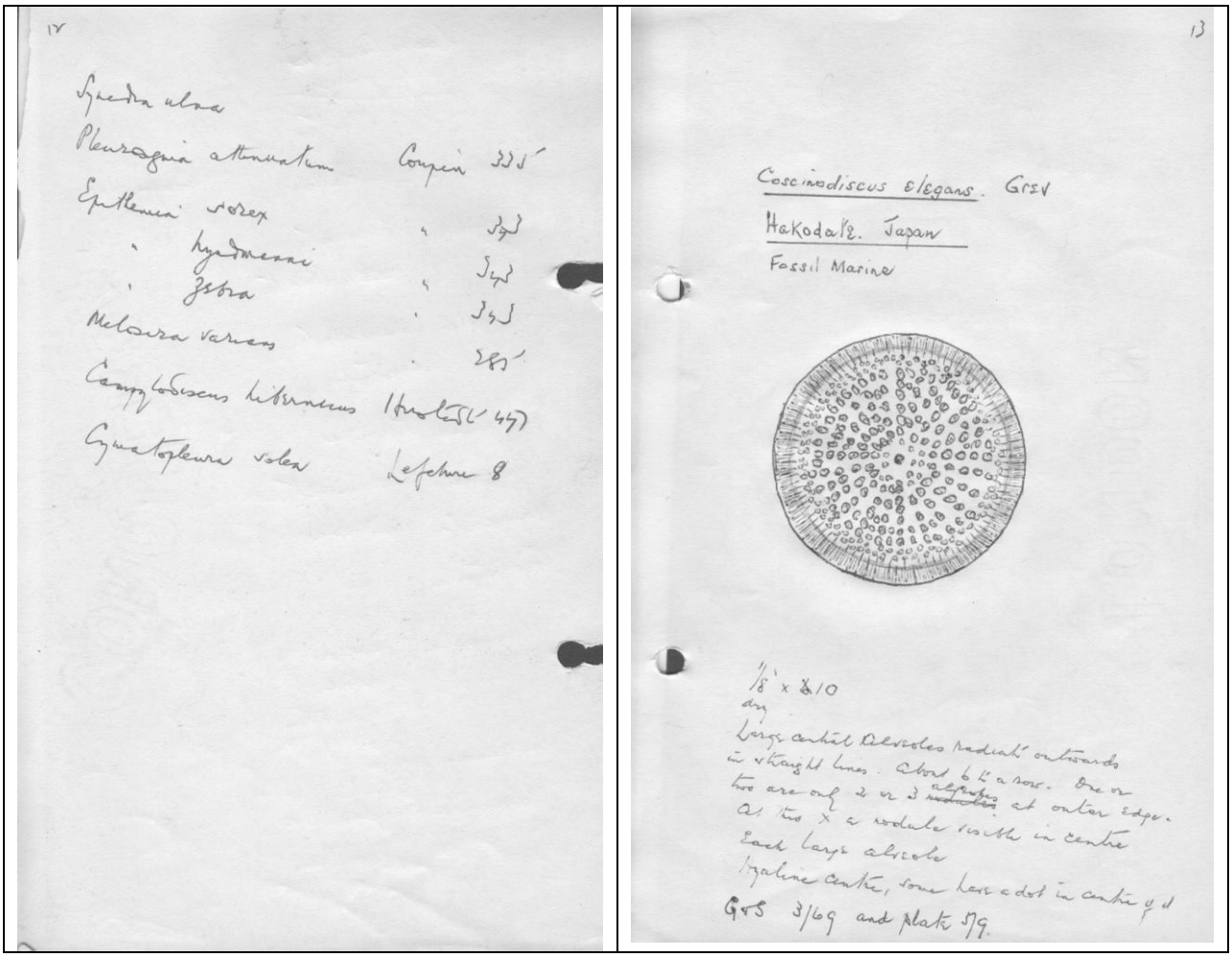
Planktonella Sol

Per Lauterborn Portmoral - Pl. III

11

Loch Cuithir
Isle of Skye
Per N. L. Flatters.

Cymatopleura elliptica	Compare	350
Surirella biseriata	"	352
" ~~Pand~~ robusta	"	351
Cymbella lanceolata	"	341
" tumida	"	341
Epithemia turgida	"	343
Stauroneis phoenicenteron	Lefebvre	XXVI
Navicula radiosa	"	XXVIII
Cyclotella sp		
Navicula illumata major		
" nobilis		
" dactylus	See N plate	
Epithemia gibba	"	
Navicula stauroptera elliptica	See N plate	
Cyclotella comta	Compare	289
	Lefebvre	5



IV

Synedra ulna

Pleurosigma attenuatum Cooper 335

Epithemia sorex " 354

" hyndmanni " 354

" Zebra " 354

Melosira varians " 285

Campylodiscus hibernicus Ehrenberg 447

Cymatopleura solea Leffelmann 8

13

Coscinodiscus elegans. Grev

Hakodate. Japan

Fossil Marine

1/8" x 8/10

dry

Large central alveoles radiate outwards in straight lines. about 6 to a row. One or two are only 2 or 3 alveoles at outer edge. At two x a radiate with in centre

Each large alveole

hyaline centre, some have a dot in centre of it

Grs 3/69 and plate 79.

15.

Oamaru

A group of Triangular forms by R. G. Platten

Triceratium

undatum		
venulosum	see page 9	
Jobreanum	" " 5	Gord 19/15
Kinkerianum	" " 7	9246
pseudonervatum	" " 7	S.1163 S.2009 Row 4/8
castellatum	" " 7	S.2009 Row 4/9
	"	9203
Nova-Zealandiae	" " 6	9062
favus.		S.2009 Row 3/4.5.6 9026 9025
" Var fimbriatum	" " 8	S.1145
Morlandii		S.2009 Row 4/4
Crenulatum	" " 7	S.1142 S.2009 Row 3/10
castelliferum		S.2009 Row 6/5
capitatum	" " 9	

Trinacria ~~[illegible]~~

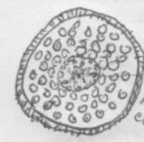
ventricosa	" " 9	9030 9103 S.2009 Row 5/1.2

16/

Jewell. Maryland

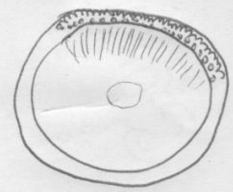
Per R. K. Platten

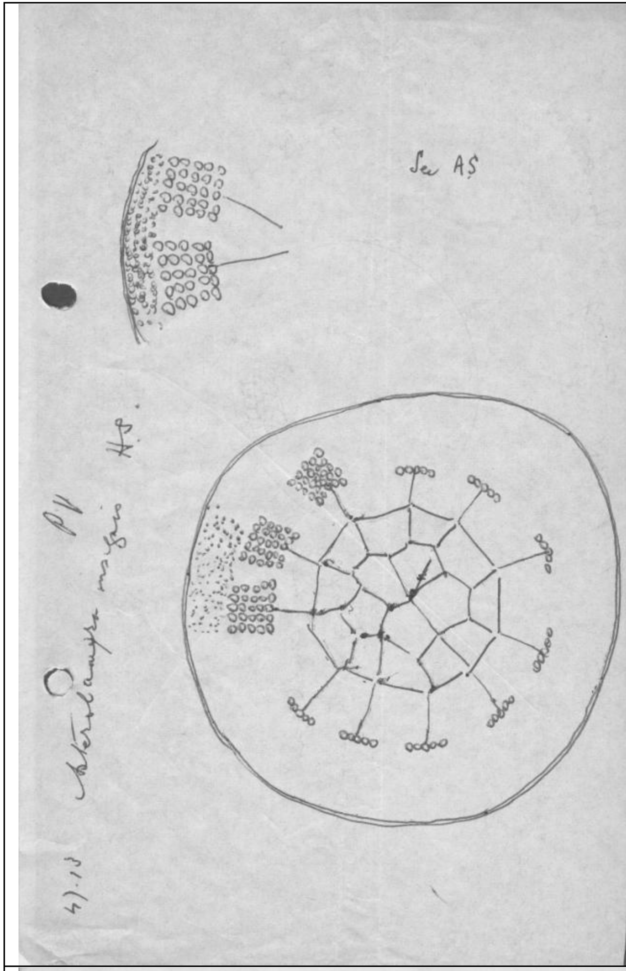
Craspedodiscus coscinodiscus

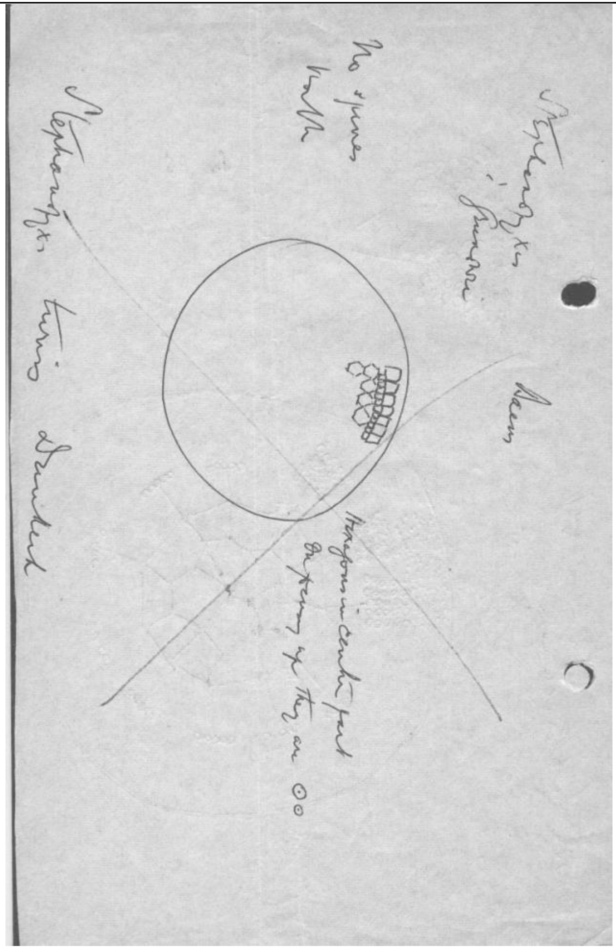


Euodia Marylandica

Melosira ornata







15

Ideas

Per W. Smith

Bohemia – v[?] to a good district. I p xii

I p xii "The City of Richmond in Virginia is said to be built upon a stratum of Diatomaceous remains 18 feet in thickness".

Used for polishing metals. A material known as

do. Tripoli derived from Ds its value as a polishing metals

do Guano is good material for marine forms

I p xiii See his note on Guano & Cereals g.' on the values from Ds.

" xiv Free Ds. eg Nitzschia. Navicula

Adherent Ds eg Epithemia, Cocconeis.

Aggregation.

a Ribbon like filament indefinite length – Fragillaria

b Zigzag chain, cohere of angles. Grammatophora Diatoma

c Gelatinous pedicel or stipes constituting of those with or particles or a fungus body

eg. Synedra. Gomphonema

d Enclosed with multitudes of confervae in a gelatinous or membranaceous matter

eg Schizonema; Encyonema or

16

Per W. Smith

Comparisons

Surirella constricta

Pl VIII 59. Valve view

V p XV 59 b Self division well advanced

59 d Self divn just completed

Pl XVIII ~~165~~ Pinnularia lata

167 a Valve view

d Self divn just completed

V p XV " Pinnularia distans

169 a Valve view

b Self divn advanced

" Pinnularia alpina

168 a Valve view

b Self divn advanced

XXVII Gomphonema geminatum

236 a valve

d Self divn just completed

p XV b " " advanced

Valves Circular. ? can be a sort of Stephanopyxis

p XVI 2 frusts close together

Or Coscinodiscus Actinocyclus

XVII Transition forms or Isthmia enervis or nervosa with cell structure of a plant or vegetable or animal tissues

17

Per W. Smith

Folly are modifications of cellular tissue :-
moniliform striae of Epithemia, Navicula &
Circular markings Coscinodiscus excentricus Pl. II fig 38
Irregular star like structure Eupodiscus argus Pl. IV 39

My Brownian motion
p xxi He ascribes it to "granular particles of the
endochrome" & says "British Desmidieae"
"Preface p xxi"
Also the chemical comp^n of endochrome

p xxii Movement of diatoms
Sect IV See his explanation p xxiv
I don't agree his description of movement
being haphazard, & non avoidance.
See my notes at when on holiday

p xxiv. Self Division Sect V
Given in many plates
Pl XV Amphiprora constricta
No 126 a Valve
d Self div^n just complete
xxii Pleurosigma Pat[illegible]
216 a Valve
d Self div^n completed

18

Per W. Smith
Classification
p xxviii Ehrenberg used ventral & dorsal sides
Kützing primary secondary sides
Ralfs Front view (= present girdle view)
side view (= valve view)
Smith followed Ralfs.
He divided into Tribes & sub-tribes
mainly on both structure & mode of growth.

Mounting
xxxii He says Balsam obscures some [illegible] markings
∴ they sh^d be mounted dry when can be seen
So I sh^d compare Dry & CB & St

xxxiii Sources of supply
Stomachs of deep sea fish.
Crab. Lobster, Sole Turbot scallop &
Whelk

Vol 2 Sect VIII Conjugation & Plates A [illegible]
Sect IX Vol 2 As Plants
Sect X " Determination sp X
xxiii " Compare forms from diff^t localities
& look for differs
Sect XI " Distribution & uses

19.

Rattray "On the Genus Actinocyclus"

Myvatn

Diatomite

In talk given by (Jeffery Harrison
L.B.O.C. on 16. 11. 65 (on "Icelandic Birds"
He spoke of Lake Myvatn & said
"Survey work is now in progress for the
establishment of a diatomite factory with an
annual production of 20000 tons which
will be extracted from the lake bottom."

FRAGILARIOPSIS ANTARCTICA (Castracane). Hustedt in Schmidt.

Cells sometimes single, but usually united to form long ribbon-like chains. Chains straight. Valves slightly convex, sometimes flat, elliptic-lanceolate in outline, apices rounded. Valve surface furnished with two systems of markings. The main structure of the valve consists of a grill or framework, in the form of several, usually 5-30, stout bars, arranged transapically, which connect with a strong marginal valve-rim. Between the transverse bars, and upon a lower plate, are numerous puncta, usually arranged in two parallel lines. The connective zone is narrow and simple. The cell is strongly siliceous.

Chromatophores: 2 elongated plates, lying close to the valves, nucleus central.

Transapical axis: 6–14 μ

Apical axis : 20–80 μ

One of the most common Antarctic diatoms, often found in enormous quantities, probably oceanic. It is liable to considerable variation in size and shape, and in the number of transverse bars upon the valve surface. P.T.O.

Taken from:

HENDEY, N.I. 'The Plankton Diatoms of the Southern Seas' Discovery Reports vol XVI cup 1937 Pg. 332 Pl. 13 figs 11, 12.

Other Refs:

HUSTEDT, F in SCHMIDT, A 'Atlas der Diatomaceenkunde' 1913 Pl. 229 figs. 9-14

CASTRACANE, A.F. 'Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873-76' Botany 2. Pg. 56. Pl. 25 fig. 12.

KARSTEN, G 'Das Phytoplankton der Antarktischen Meeres nach dem Material der Deutschen Tiefsee-Expedition 1898-9.' Deutsche Tiefsee-Exped. II Teil 2 Pg. 122 Pl. 17 fig. 7

Diatomees du Monde Entier
Collection
Tempere et Peragallo
2^e Edn 1907.

Collections

Les Diatomees du Monde Entier (2 Edn) se composent de mille préparations ~~étendues de~~ étendues de récoltes et de depôts de tous les pays du monde avec texte analytique. Cette collection catalogue 266 genres dont 6 nouveaux et 6059 espèces ou variétés dont 347 lui sont propres -
Prix de la collection complète 1000 fr
(Nota. Trois collections seulement sont disponibles)

Sold by Chez J. Tempère, villa Andrée-Lucie à Arcachon (Gironde)

1000 diff slides complete with text £50!

Notes from S. H. Meakins correspondence

Guide lines. Use a 00 sable hair brush fixed in the cork of a bottle. Draw brush to a fine point on edge of glass, making sure glass has no sharp corners. Put a drop of ink on a slip of glass & dip brush in ~~that~~ ~~[illegible]~~ ~~[illegible]~~

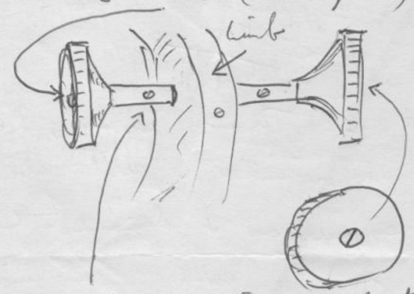
you will make the adj. too tight. Let me know how you get on - you must get a good screwdriver otherwise you will spoil the screw heads.
Yours, Eric

368, Cherry Hinton Road,
Cambridge,
13/9/67.

Dear Mr. Catalani,

Thanks for your letter, I have been intending writing about the angle eyepiece, as I decided to hang on to it in the end, as it incorporated in a small portable microscope I made during ~~the~~ summer.

It will be a very simple matter to tighten the coarse adjustments on the 'Service' stand. I shouldn't touch the slide adjustment as I don't think there is any play in ~~that~~ at all. Look at the two coarse adjust wheels and you will see a screw in the centre (see diagram)



On the shafts you will see a tiny grub screw each side - what you should do is loosen these carefully with a tiny watchmakers screwdriver (not too much or they will drop out) & then tighten up the screws in the fronts of the wheels very slightly with a suitable screwdriver. Make sure you get a screwdriver that fits these screws perfectly & I think if you tighten them (holding the opposite wheel) this will do the trick - do it carefully otherwise

Authors who have named Diatoms.

[This listing is by no means comprehensive]

Arnott, George Arthur Walker 1799-1868
Azpeitia Moros, Florentino
Bailey, Jacob Whitman 1811-1857
Barker, John William 1887-1948
Brigger, Albert L
Brun, Jacques 1826-1908
Cheneviere, E
Cleve, Per Theodor 1840-1905
Cottam, Arthur -1911
Ehrenberg, Christian Gottfried 1795-1876
Forti, Achille Italo 1878-1957
Fuge, Dingley P
Grant, William M
Greville, Robert Kaye 1794-1866
Grove, Edmund
Grunow, Albert 1826-1914
Hanna, G. Dallas 1887-
Harvey, William Henry 1811-1866
Hustedt, Friedrich Carl 1886-
Janisch,Carl 1825-1960
Johnson, Christopher
Kitton, Frederic 1827-1895
Lefebure, P
Long, John A
Mann, Albert 1853-1935
Meakin, Samuel Henry 1876-1955
Norman, George 1823-1882
Pantoscek, Jozsef 1846-1916
Peragallo, Hippolyte 1851-
Ralfs, John 1807-1890
Rattray, John 1858-1900
Schmidt, Adolf Wilhelm Ferdinand 1812-1899
Smith, James
Sturt, Gerald 1860-1947
Tempere, Johannes Albert 1847-1926
Walker, W.C.
Wise, Frederick Clunie 1884-1962
Witt, Otto Nikolaus 1853-1915
Van Heurck, Henri Ferdinand 1838-1909
Möller, Johann Diedrich 1844-1907

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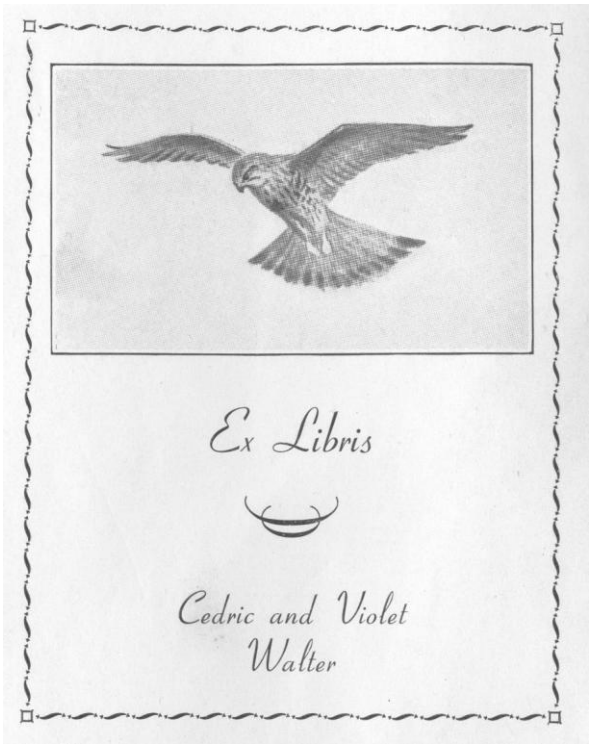
APPENDIX A

PMS Box. No. 357



No. 12
OPERATION DIATOMS

by
Cedric Norman Walter
"Rotherwood"
32, Stanley Avenue,
Beckenham.
Kent.

May 1965.

Contents

INTRODUCTION

In January 1963, I sent round a box with a little dissertation on, what is to me, “The Fascination of Diatoms", and in my Foreword I said:-

> "The Diatom slides I am sending round now are by other mounters, some by the masters, as a tribute to their work, the examination of which has given me many an enchanted hour. Now that I have a little more time, I have taken up the mounting of diatoms - but that is another story.
> I hope to tell you about ‘Operation Diatoms’ in a later contribution."

Well, my friends, here it is. This time you will not get the work of the Masters, or a Master, but only the humble effort of one who is trying, rather falteringly, to follow in their steps.

Most of us like a Microscopical Gossip. It is always interesting to hear how the other chap does it, so you may not find uninteresting the following notes on my pilgrimage through this form of mounting. Perhaps it may encourage some others, who have not already done so, to take up this most fascinating game of making diatom preparations.

The mounting of diatoms certainly opened up to me a new and wonderful experience in the world of Microscopical Preparations as I soon discovered

At Belstead we had learnt how to make fluid mounts and dry mounts of other subjects and these principles could be applied in the case of diatoms; but to my idea, that is not the real art of diatom mounting. My ambitions went beyond that. The mounting of diatoms is not taught at Belstead, the reason being, as Mr. Marson explained, that so few people were interested, which is true.

So the question was how to find out the way the real mounting was done. Diatom mounters to-day are relatively few and far apart; there are none anywhere near me from whom I can get any practical and personal instruction, so I had to set forth to ‘furridge’ it out on my own from books or periodicals.

I managed to get hold of the copies of two lectures which must have been given many years ago. One by E. Leonard, "A Paper read before the Liverpool Microscopical Society"; and the other by Sidney Chaffers, "A Paper read before the Manchester Microscopical Society". There is no date on either but the indications are that in each case it must have been more than thirty years ago. Each consisted of 11 quarto pages close typed, and by the time I had read through these I felt like abandoning the whole idea, so meticulous and detailed was the procedure; however, I had accepted the challenge and was in honour bound to carry on.

My next step was to analyse each paper right out to get a complete programme and order; that in itself was quite a task; but it did do one thing, I began to get a grasp of the principles of making these preparations.

Then I was fortunate. For just after that, in looking up a pre-war volume of "The Microscope" which I had borrowed to refer to something else, I came across the series of articles written by S.H. Meakin and C. Swatman. This was another complete and well detailed description of the art. It all seemed very complicated and did not seem to agree with the other two papers mentioned above. However, again I analysed all these articles in the same way, and when I came to compare the three I found that all worked on the same general principles and the differences were only those in detail and method. One finds this with other forms of mounting.

But one thing was outstanding in all of them and became thoroughly impressed on my mind, namely, the need for the strictest attention to every detail and above all cleanliness. In due course I found out why. One does not know the meaning of dust and dirt until you mount diatoms; or the need for filtering everything used of a liquid nature. Of course, you can just put a bit of adhesive on a slide, put a diatom on it, some mountant on and a cover glass and say, "That's that, what is all the fuss?" One might get a lucky one, mostly not, just a mount of a sort.

It was certainly not the way of the Masters. To my mind it is much more satisfying to carry it out thoroughly and try to produce a mount one can be proud of in every respect. I am afraid I agree with the perfectionists, of whom S.H. Meakin was one.

I decided to work more or less on the methods set out by S.H. Meakin; I had the proof of it in the many of his beautiful mounts which I possess, also he was nearer to to-day, (he only died in 1955) and the re-agents he mentions were more likely to be obtained to-day than some of those given by the earlier mounters.

I will not bore you with all the difficulties I met with and the many frustrations and failures through lack of any personal tuition, or the lack of the little tips that make all the difference; suffice it, that I gradually worked out a programme which I find produces results and it is the method very close to that of Meakin. .I am, indeed, very indebted to him, so skilled a mounter, for publishing his methods that others might benefit.

Meakin used one Microscope with a Mechanical Finger. I use two Microscopes for some mounts. You see, in the beginning, I did not have a mechanical finger and did not know where to obtain one, but I have a Watson Research Microscope which is binocular and stereoscopic. It is low power, maximum is X140. It has a fine large moveable stage, plenty of working room at all magnifications, and arm rests; it really is a lovely instrument and one which I have always enjoyed using for many purposes.

One, to me, little problem was how to get the diatom from the Search Slide, where they are all jumbled up together and dry, on to a mount slide in a particular position, and I wanted the two slides close together; so I made

a small frame and fitted it on to the stage of the Research Microscope and thus held the two slides parallel to each other. I had three cat's whisker bristles of different thicknesses set in penholders, which were very useful for arranging delicate parts of insects and I found I could, by hand, pick up a diatom from the Search Slide with one of these and place it on the adjacent mounting slide - that is I could do so after some practice, it was not easy at first. It was equally useful where a diatom is dirty and wants washing. Using one of Mr. Horrocks' hypodermic syringes, I can put a tiny drop of distilled water on a slide, pop the diatom into it, wash it and take it out again and place on the mount.

This hand method worked quite well if it was a single diatom which did not require positioning in a certain way, e.g. a *Triceratium* where one wants the base of the Triangle level with the edge of the slide. To do more than one diatom and get them into the right lay-out was a matter of luck although my hand is pretty steady - sometimes I succeeded and other times I did not. This was going to be a drawback.

I realised, however, it would be possible, by hand, to get two or three diatoms level provided I had a guiding line, and I cannot use a Graticule in the eyepieces of the Research. Then I made a discovery. I do not know whether other members know it but with the ordinary ninepenny Biro one can draw lines and circles on a glass slip provided it is grease free, and a mounting slide must be that. The Biro marks can be easily removed later with a little Rinso. So, by drawing on the under-side of the mounting slide a horizontal line across the centre of the slide and making a tiny ring on the ringing table in the centre of the line an excellent guide is provided, which is quite clearly seen through the slide under the Microscope. Provided the diatoms were not too close together I could now place them in a line. But this was going to be sufficient for more advanced preparations.

Then, one day, I saw an advertisement in "The Microscope" by R.I. Firth of Lewes, of a Mechanical Finger. It was a lot of money but it was a copy of the one Meakin used, so I had it fitted to my Watson Service Microscope. I found it was not quite so simple, to me, as it sounds in Meakin's article and some time and practice were required. Such things as knocking the holder, or racking down the objective too much, or forgetting to raise the bristle when changing a slide. One day I broke the tip off the bristle and a nice old game it was trying to make another one out of celluloid like the original. A Cat's whisker did not seem to work successfully and for a time I did not get much use out of the Finger and did all I could by hand. Then a fellow member of the Quekett who, strangely enough, does not actually mount diatoms, recommended the use of Entomological Pins, and this ended my troubles and put the Mechanical Finger back into operation again. I use them now for hand work. These pins can be obtained with very fine points and variable thicknesses. The finest point can easily be slightly widened by very lightly drawing across a piece of wet-dry emery paper once or twice.

So I do most of my mounting by hand, except in the case of a group or where very fine adjustments are required, when I place them roughly in position by hand and finish them with the Mechanical Finger. The Mechanical Finger is all right if you can have the two slides side by side, and I believe the big mounters had special stages made for the purpose. The stage on the Service is not large enough for two slides, and to keep changing them each diatom not only takes extra time but considerably adds to the risk of damage as I have experienced.

As I have mentioned in previous note books, I keep a Microscopical Diary and in it I note, among other things, all my difficulties, and otherwise, while actually making the preparation, ending with comments on the final result. It contains complete details of each mount so that I can always refer back later and know exactly how it was prepared. I have a separate Diary for Diatoms and it is interesting now that I have made some progress to read over the notes and comments of the early struggles.

As I have said; I do not propose to bore you with the details of my various and so frequently disappointing diatom mounting experiences, but just to deal with, and illustrate by the mounts herewith, the various aspects of the art and the methods I have adopted. If anyone would like to have any further details, or to discuss any points I would be only too happy to send them, discuss them, if they like to write to me direct. I am always pleased to hear from a fellow member.

To my very great regret, I cannot say I clean any material, either fluid or fossil. I know how it is done but I have not ventured on it. The reason is that I feel I cannot take the risk, at my age, of handling the acids which are required. I might become over anxious and have an accident with them; so in these circumstances I bow to wisdom and leave it alone. Happily, I have been able to acquire from various sources a nice little stock of well cleaned material.

Now for the Mounts.

<u>SLIDE No. 1. Fluid Mount.</u>

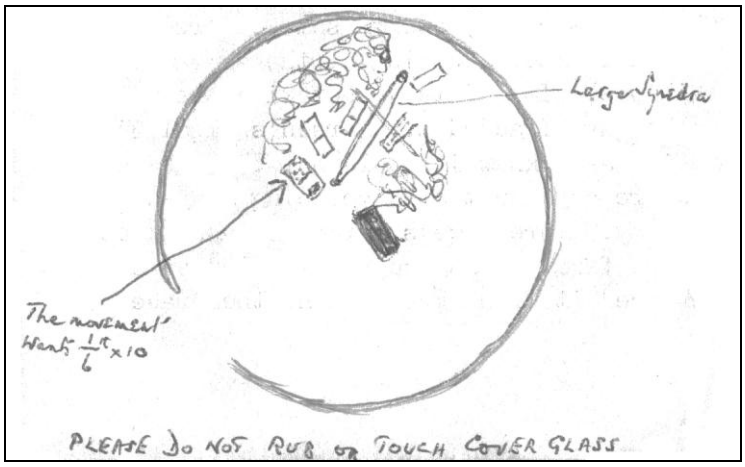
This is the most simple mount, and one that anyone can do who is interested in Pond and Water Life. It shows the Diatoms in their natural state.

The material was collected from the River Otter in Devon, and reminds me of a· most delightful morning which my Wife and I spent at the beginning of October 1961. A lovely warm sunshiny day, and we waded about in the shallow parts of the River taking scrapings from under stones and squeezing out submerged plants. Later, under the Microscope we found we had been rewarded and there were plenty of diatoms of several species. When we

got home, my Wife let me have a fine-mesh coffee strainer which I used for the purpose of getting rid of the debris. Unfortunately, I did not then know the technique and a proportion of the diatoms were lost, so this is not fully representative of what we gathered.

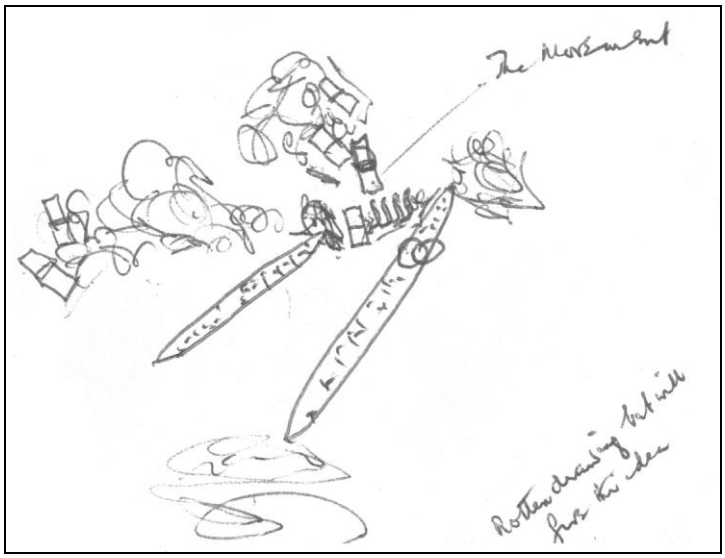
There is, however, a remarkable feature about this mount, and in some others I made from the same gathering at the same time as this one. Immediately on taking the gathering, I added Mr. Marson's Algal Fixative to it, as I knew I should not be able to prepare any mount at once being away on holiday. Three weeks later, I washed out the fixative as taught at Belstead and replaced it with 1% Formalin, then made the mounts. There is BROWNIAN MOVEMENT in some of those mounts and in the one now before you. In another mount, done at the same time, there is a *Melosira* which is so full of particles jumping about that it looks like a boiling cauldron. I cannot risk sending that one, it is so exceptional and I want to see how long it will last; like this slide, it is still going strong after 4½ years.

However, on this slide No. 1, I have placed a small red ring on the cover glass and inside that circle at about just above centre you will find a frustule in a position like this:-



Being a fluid mount, the formation may change. It will be affected by shocks in transit and also because the slides do not lie flat, in this packing. A *Navicula* now close to the frustule with the Brownian Movement, was on the lower side of the left *Synedra* two days ago!

At November 1966, what to look for is as shown hereunder, a little to the right of the centre of the red ring, and the movement will be in a *Melosira* frustule.



It has a number of particles all moving about. Well, they are there now and have been all the time, but what the effect on it will be of the jolting about on circuit, I cannot say. It will be interesting to know.

But the problem is, what are these moving particles? Are they granules of protoplasm? If so, why have I never seen anything of the same nature in fluid mounts of other gatherings? There is another possibility that occurs to me. This gathering stood in Algal Fixative for three weeks and the formula for it is, I understand:

Nickel Acetate 2 gms
Copper Acetate 2 gms
Distilled Water 100 mls.

When it is used, it is added to the extent of one third of the gathering. Would these minute particles be connected in some way with either of the Acetates?
The method of preparation was the usual one for fluid mounts.

SLIDE No. 2. Strewn Mount.
This is, I suppose, the simplest form of mounting cleaned diatoms. The diatoms are kept in a tube of distilled water. One or two drops let fall·from a pipette on to a cover glass. Heat the cover glass to dry out. Put mountant on slide and invert cover glass over it. These mounts can be done quite quickly and will give an idea of the nature of the diatoms one can expect to find in that material. If one wants to see a particular diatom again, one must use a marker, or a finder, or take Vernier readings.

SLIDE No. 3. Adhesives Comparison.
As with sections, an adhesive is necessary to keep the diatom in position on the mount. When I first started, the only adhesive I had was Glycerin Albumen which I had used for sections. I found it worked quite well for diatoms, just the faintest smear and, as I learnt later, it is better to filter it twice or three times before use. Most of my mounts are done with this adhesive.
Meakin gives a formula for Dextrin but I could never get it to work properly, it was uncertain. Whether it was that my proportions were wrong, or I had the wrong Dextrin powder, I do not know; anyhow I do not use it.
One day I came across a formula for Gum Tragacanth. This I made up and filtered three times. I find it all right if it is just one or two specimens on a·mount but not for larger numbers. Rather a job to keep it viscous, after breathing on it, to move the diatoms. Further, it seems to develop mould in the bottle and does not keep too long; Glycerin Albumen retains its viscosity for several days.
On the slide now before you are two mounts, side by side, for comparison, with exactly the same diatoms, in the same order, and in the same mountant. I have particularly chosen diatoms of a fine structure and not too easy to resolve. They are from a pond, at Retford, Notts., and reading from left to right as you view them (title to the right)they are *Surirella, Pleurosigma* and *Cymatopleura*. They are very fragile diatoms and rather tricky to mount. When doing each mount I had a breakage on the mount slide when moving them into position and had to try to remove all the bits, and this will account for some of the specks you see, which were too small and too firmly fixed in the adhesive to remove them.
With the title to the right, the adhesive in the left hand mount is Glycerin Albumen and in the right hand it is the Gum Tragacanth. I cannot see there is any difference between them; you can move from one to the other without altering focus.
When first put on the slide, the smear of fixative can be seen if one holds the slide to the light. After the diatoms are in position and have been on the hot plate the adhesive evaporates or is burnt out, and if the slide is held to the light the glass then clear. But some of the adhesive must surely remain under the diatom or it would not stick so firmly. It would be under the edges of some diatoms or under most of the valve if it is a flat one. Does this have any effect on Refractive Index. We worry ourselves a good deal about the R.I of the mountant, but what of the adhesive?

The formula for Gum Tragacanth was :-

	Quantity	**R.I.**	**Proportion R.I.**
Gum Tragacanth	1 cc	1.512	0.01512
Distilled Water	99 cc	1.554	1.5206
When dissolved, Phenol	3 cc	?	?
			1.335

I do not know the·R.I. of Phenol or whether it would affect the above.
The Glycerin/Albumen I use was purchased from George Gurr's in liquid form so I do not know its R.I. But in Peacock (2) is given a formula for it:-

	Quantity	**R.I.**	**Proportion**
White of Egg	50 cc	1.550	0.615
Glycerin	50 cc	1.475	0.756
Salicylate of Soda	1 gm	?	?

1.411

The proportion of White of Egg, equal to the quantity of Glycerin seems high to me.
To arrive at the R. I. of the two adhesives is it right to divide the R.I of each ingredient in the same proportions as the formula, as I have done above, resulting in:-

Gum Tragacanth	1.335
Glycerin Albumen	1.411

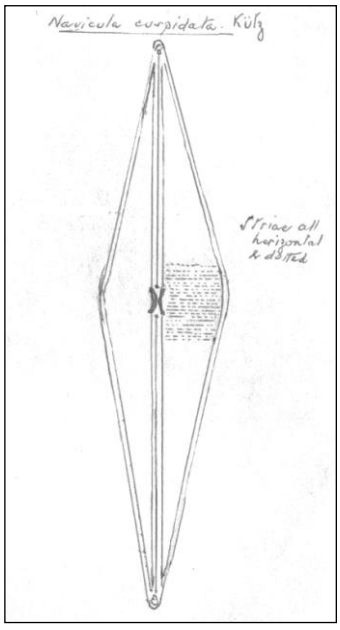
Also, I presume, we must consider the R.I. of the Diatom itself in this comparison; the closest to it above is the Glycerin Albumen. I regret to say I know nothing of the intricacies of Refractive Index calculations. Some of you my friends ·will be able to sort this out - if, in this case it really matters.
(Handwritten Note: R.I. Diatom Silex =1.434)

To make comparative mounts of this kind I drew, with a Biro, a line and the rings on the under-side of the mounting slip, as described previously.

MOUNTANTS.

Slides 4-6

Here is something more for the R.I. experts. Eighty years ago, Canada Balsam was superseded by Styrax for mounting diatoms; because it had a higher R.I. and gave better definition for fine structures. This has been followed by several other mountants of variable R.I.'s ranging from the Canada Balsam with its 1.526 up to Realgar 2.549, but the commonly used and proven medium is still Styrax.
In my ignorant way, I have wondered sometimes how much there really is in this search for higher R.I for diatoms, first bearing in mind that so many of the detailed studies of diatoms were done in the last half of the last Century (and how admirable they were} when mounts were in either Canada Balsam or Styrax. Think back to W. Smith, Grunow, Kitton, Rattray and many others. When you come to check their observations with mounts of their period it is remarkable what they were able to determine.
My limited experience has shown that some differences must be due to the diatom itself. I have one or two mounts, prepared in my amateur way, where, comparing the same species, the one mounted in Canada Balsam is far more brilliant in its appearance and in its detail than the same species mounted in Styrax. Here, I am not speaking of quite such delicate structures as those appearing in the mounts Nos. 4-6 you are about to see, but in more robust species.
But, you will notice some of this variation on these slides 4-6, nevertheless, and that the resolution of *Navioula cuspidata* will vary quite a bit in a mount. This may be due to slight differences in structure or formation, or whether the diatom itself is lying perfectly flat. It might also be partly due to the setting-up of the Microscope; diatoms are a great test of this.
I have made some special mounts for comparison of the mountants which I have so far used, or tried to use. The diatom material chosen is the same for each, viz. strewn freshwater diatoms from a Pond near Harlow, Essex, gathered and cleaned by our fellow member·Mr. H.J. Hall, who was good enough to send me some. I thought it would be very suitable for these comparisons, because the markings are very fine, particularly that of *Navicula cuspidata*, which is the dominant species in the mount. If a comparison is to be a true one then one must compare like with like, and the same species of diatoms should be used·and from the same gathering, so that the only difference between the mounts will be the mountant, and this is what I have attempted to do in slides 4, 5 & 6.



You will observe that against each R.I. I have given the increase per cent of that R.I. over that of the other mediums. The point I raise about this is, should one see that much more increase in appearance and resolution? If this is not apparent, why use, the mountant? If you are like me you will not see so much improvement as the increase in percentage seems to indicate.

SLIDE N0. 4. Dry and Canada Balsam.
To begin at the beginning, this first comparison is between a dry mount and one in Canada Balsam. These two were made at the same time, drops from the same pipette dip, on two cover glasses, which were heated and dried together.

With title to the right, the left hand mount is the dry one and the right hand one, in Canada Balsam. Note the contrast and how much clearer the details of *Navicula cuspidata* are in the dry mount than it is in Canada Balsam!
Does the thickness of the mountant have any bearing on this? This thickness is evident because after travelling along from the dry mount to the balsam mount, one has to rack up to get into focus.
Perhaps I should mention that I always use No. 2 Slide and No. 1 Cover Glass. The No. 2 slide because the Service Microscope is adjusted for that thickness according to Watson's.
The method for the Dry mount was, first a ring of Murrayite and when tacky, one of the cover glasses inverted on to it, pressed down and then ringed in Murrayite. The other mount was the normal strewn method given previously.

Slide No. 5. CANADA BALSAM, STYRAX, NAPHRAX

These three strews were all made at the same time out of the same pipette and from the pond at Harlow as in the previous slide.
The R.I.'s are:-

	R.I.	Increase
Canada Balsam	1.526	
Styrax	1.583	Over C.B.3.95%
Naphrax	1.76	Over C.B. 15.79% Over Styrax 11.39%

Naphrax is, I find, a bit tricky owing to bubbles. Sometimes I'm lucky and sometimes not. The solvent for it is Toluene; it is difficult to get hold of as it comes from the U.S.A. A small quantity was on offer at the Quekett last year and I was able to obtain a little.

SLIDE NO. 6 STYRAX, CLEARAX, NAPHRAX.

These diatoms are from the same source as the two previous slides and made in the same way.
The R.I.'s are:-

	R.I.	Increase
Styrax	1.583	
Clearax	1.666	Over C.B. 9.21% Over Styrax 5.06%
Naphrax	1.76	Over Styrax 11.39% Over Clearax 6.02%

I have only used Clearax once or twice, a trouble is bubbles, also, although I have filtered it, it soon turns white and cloudy in the bottle. I wonder ,whether this is mould.
Incidentally, it is better to filter all the liquid mediums before using them to mount diatoms.
There are, of course, several other mountants, which I have not yet used, although I have them, but all being well, I hope to deal with them in a later contribution after I have tried them out.

SELECTED MOUNTS.

These are the more difficult forms·of diatom mounting and take time and require great care in preparation. My method is as follows:-
Preparatory.

Mounting slides, made grease and chemical free
Mounting Slides. Put guide ring or line on the under-side.
Cover Glass. Made grease and chemical free
Cover Glass. Put guide ring in centre.

Procedure.

(a) A few drops of distilled water containing diatoms on Search Slide and left to dry.
(b) Pick out desired diatoms.
(c) Wash individually those with any dirt attached.
(d) Adhesive on mount slide.
(e) Diatom, or diatoms, displayed on mount slide.
(f) Heat on Hot plate.
(g) Clear in Xylol for Canada Balsam, in Benzene for Styrax.

(h) Mountant on.
(i) Cover glass on with guide ring inside.

Those are the stages but there are many details which require careful attention in each one. I will not put them in full here, but if anyone wants to know more about them, or discuss them, just drop me a line direct. I shall be pleased to hear.
For Multi-slides, that is more than three or four, I use Canada Balsam for the reasons given a little later.

SLIDE No. 7. One Aspect.
The conventional way of showing a species is the valve view of either one or more of the same species. In this instance here are four valve views of *Arachnoidiscus Ehrenbergii*, from False Bay, South Africa. This mount was done by hand only, which accounts, for one of the diatoms being slightly out of balance.
Where a diatom is plentiful it is I think, better to do three or four of ' the species in the one mount, because there are sometimes slight variations, and detail is sometimes more visible in one than another. I now mount a single specimen only when the diatom is scarce.
The adhesive was Gum·Tragacanth.

SLIDE No. 8 Two aspects.
One does not often come across diatoms mounted purposely in this way, that is, to show both the inside and the outside view of the valve. Here is *Stephanopyxis Grunowii* from Oamaru. The smaller one is outside view and the larger is the inside view. This is very clearly shown under a Binocular Stereoscopic Microscope. Under the Monocular the distinction is apparent by the difference in focus; rack up to get the hexagons clearly shown in the centre part of the smaller and compare it with the other one; rack down for the larger one to get a similar view and comparison. In some diatoms like this one, there does not seem to be any marked difference, but it can be more apparent in others.
Adhesive - Glycerin Albumen.

SLIDE No. 9. Four aspects.
As a deliberate form of mount this one is, in my limited experience, rare; but it should, I suggest be the ideal, because it shows the diatom in all its aspects. The reason for the rarity of this lay-out is, no doubt, the difficulty of obtaining four diatoms of the same species for display in this way. One finds the valves but it is the complete frustule which is scarce.
This is *Trinacria insipiens* ? and is from Kamischev, Russia, and I checked these four carefully with high power before mounting to make sure they were all exactly alike in valve view and the same species. With the title to the right you will see:-

On the left. The outside view of a valve, processes down.
Next to it. The Girdle view of the complete frustule, showing .how the valves are joined together.
Next. Zonal view of a valve
Then. Inside view of valve, processes upwards.

I have still to get this four aspect display with a discoid form, not only to find a complete four, but to mount the zonal and girdle view. The only way to get these two views securely mounted is to chip a piece off one edge to get a straight edge, and then stand it vertically on that. Far easier said than done.
Diatoms are, at times, cussed things. Occasionally, when doing a multi-mount, when I want only valve views, a discoid will rise up on meeting the adhesive, balance on its edge and refuse to go flat, but it would not be much use like that unless one has the valve view to compare it with and identify it. When I want it to·stand up on a species mount it refuses to stay put. In this mount the diatoms were placed roughly in position by hand and then gently put into place with the mechanical finger and aligned with the eyepiece graticule.
Canada Balsam was purposely used because I did not want to risk spoiling the display of the vertical ones. I find that with Styrax there is more chance of this occurring. First the rushing movement of the Benzene used for clearing when first put on, and if it survives that, there is the more violent movement of the, or of my, Styrax, which lasts for several seconds and then dies down gradually. So I do not risk selected multi-mounts with it and use Canada Balsam which is comparatively quiet in action, also I believe it hardens better than Styrax does and less chance of diatoms becoming loose later, especially if they go on P.M.S. travels.
Adhesive was Glycerin Albumen.

SLIDE No. 10 Mystery Slide.

When one is working from the Search Slide there are to be seen numerous fragments of diatoms of various sizes, some of which arouse my curiosity. I pick a few out and mount them, as in this case, because they cannot be properly examined until mounted. and higher power can be used. It is rather fun trying to identify them and it sometimes discloses the presence of diatoms which one has not come across in their entire form in that locality. It was so in one or two instances in this mount.

These are from a Search Slide of Bain's Farm, Oamaru, and there are six or seven genera. I have had a quick go at identification and suggest the following (title to the right):-

Top Row. Left to right.

1. A very large *Triceratium*?
2. *Asterolampra insignis*
3. *Asterolampra insignis*
4. A very large diatom. Note the group of dots in each hexagon.
5. Part of one I have not seen before. ? *Lepidodiscus.*
6. ? an interloper, a Radiolarian
7. An arc of one new to me. ?*Porodiscus*
8. ?*Eupodicus*
9. Same as No. 5
10. An *Aulacodiscus* of exceptional size.

Bottom Row. Left to right.

1. Fragment of a *Triceratium*?
2. Repetition of No. 7 above
3. *Coscinodiscus lineatus* ?
4. *Asterolampra insignis*
5. *Asterolampra insignis*
6. *Triceratium* ? sp.
7. ? *Aulacodiscus*.

Diatom detectives, have a go!
Adhesive Glycerin Albumen.

SLIDE No. 11 Genus Slide.

I call this a Genus slide because it is an attempt to show several species of one genus mounted together. In this case, *Triceratium*, and a group of 10 species, from Simbirsk, Russia. I do not think, however, I was quite successful, as one or two may be the same species and one may be a *Trinacria*. I have not worked out the full identification, When the diatoms are small and the differences in the markings are not pronounced, it is difficult to·determine the species exactly until they are properly mounted and viewed under high power. On the Search Slide, even under high power, many are not clear enough and some are dirty, so some of it was guess work. This mount was done entirely by hand on the Research Model. A Biro line and ring was drawn on the under-side as a guide, but as you can see they are not quite so truly in line as they would have been had I used the mechanical finger and eyepiece graticule.
Adhesive Glycerin Albumen.

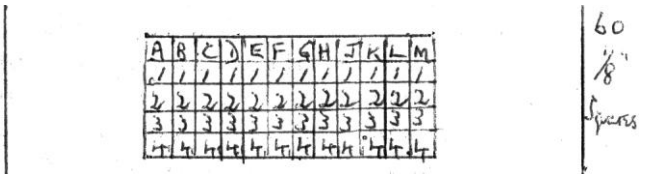
SLIDE No. 12. Type Slide. 157 Diatoms.

The diatoms are from Palos Verdes, California. Our member, Mr. H.J. Hall cleaned some of the fossil material from this locality and sent me some of the result. He certainly cleaned it very well.
Some might not call this a Type Slide because they are not all separate species on this mount, I have not been able to complete a full identification of the 157 diatoms shown, only as far as genera, of which I make it there are fourteen:-
Coscinodiscus Arachnoidiscus Actinoptychus, Triceratium, Stictodiscus Hyalodiscus, Melosira, Auliscus, Terpsinoe, Navicula, Grammatophora, Biddulphia, Cocconeis, "A.N.Other"

This mount was the result of a careful examination of seven Search Slides, the total number of diatoms on which was about 20,000.
However, I regard this as a Type Slide, because it shows in an easily referenced lay-out some of the species in their numerical relationship, i.e. Dominant, Prevalent, or Scarce, Thus, the dominant form, the most numerous here, is *Coscinodiscus*, and a number of species of this genus are given. Prevalent forms, that is those which, while not numerous, occur fairly frequently, e.g. *Actinoptychus*, of which several species are set out. Scarce, those which are

difficult to find and of which, in the main only one is on the mount - for the good reason that it would be the only one I found; one in 20,000.
This slide is laid out in columns, ten columns, and reference is by the position of the diatom in the column reading downwards, and numbering the columns left to right. (Title to the left.)

Before preparing a mount of this kind I one has some picking out to do and I systematised this to suit my double microscope method. It may well be there are other ways of doing it but this was the one I worked out.
I have ruled a number of slides thus:-



This was done with a diamond pen but could be done with a Biro. The marking is, of course, for the under-side, and shows quite clearly when under the microscope. I rule these for use on the Research which is Binocular. One must allow for reversal if used only with a Monocular Microscope.
Incidentally, using a squared Search Slide enables one to make a rough count of the total number of diatoms and work out an approximate numerical relationship.
For example, on the seven Search Slides I went over for this mount there were about 3,000 diatoms on each slide, and that was if anything, an underestimate.
Some of these ruled slides I use for Search and some for what I call Store Slides. In the Search Slide one can go over it systematically square by square, picking out what is wanted and putting it on the Store Slide, where each square will be used for a separate genus. Also, on the Store Slide the diatoms can be washed with the aid of Mr. Horrocks' syringe as mentioned, earlier. (If one were mounting wholly by Monocular one could dispense with the squares and depend on vernier readings.)
After that, the Store Slide can be examined under high power to get an idea of the species and also to see whether any are damaged. When doing that I draw a large diagram of each square, marking the position of each species, what it is - if I know it, and whether worth mounting. From these diagrams I work out a plan for the final mount.
The preparation of one of these multi-mounts is the most exciting of any form of microscopical mounting - so much can go wrong and one's effort be wasted after spending a long period of time. It is quite a thrill all the time but one has the reward of a wonderful feeling of pleasure and satisfaction when successful.
Take this present mount, according to my Diary the actual mounting of this slide was spread over 10 days with two or three extra days for picking out and planning. It is rather exacting work and at my age I cannot do too much at once. Some days the work goes quite well, each diatom conforms and goes quite easily into its place; while on another day they seem to have the very devil in them. One or two will stubbornly refuse to move on the adhesive, breathing on them and everything else, and it takes a long time to get them into the required position; or else they break when nearly there and that means another long waste of time, because all the fragments have to be removed, and don't some of them stick hard! There are one or two bits on this slide which I could not remove. This upsets the plan if there is no duplicate, of the damaged species. But these are minor troubles, for over all there hangs a Sword of Damocles in the form of the Mechanical Finger. Forget to raise it before removing the slide and the odds are it will out through the field scattering and damaging diatoms. If it happens at the beginning of a mount it may not be so bad, but when nearing the end of a crowded mount, it is a tragedy, to put it mildly.
But let us come back to this mount. The days went by and the number increased, and then the Sword fell. I had got 79 in position and forgot to raise the finger before taking off the slide. Fortunately·, it was to place it on the Research, where I at once found part of my pattern was disarranged and more than a dozen diatoms were all higgledy-piggledy. Well, was I exasperated? Imagine what you would say in those circumstances. However, I knew it would be fatal to do anything about it while I was in that agitated state of mind so I went off and left it, and found consolation in the words of Isaiah. Ch.30 v15.

"In returning and rest shall ye be saved; in quietness and in confidence shall be thy strength."

After an hour or two I came back with those words well in mind and quite calmly, and I am glad to say, successfully repaired the damage. I was indeed fortunate. There is one thing I have learnt in mounting diatoms, particularly where there are several, and that is that perfect tranquillity of mind and great patience are essentials, otherwise everything will go wrong. To one of my temperament who has always been quick and impatient and irritable when things are not right, this is a most salutary lesson.

Well, I went on. Up to 108, then 123, 136. The tension grew and the last ·couple of nights my sleep was affected by anxiety. At 157 I had completed my plan and could call that part of it a day. But .still the tension and excitement remained for all was not yet finished, the work could still be spoilt.

Would they stand the heating on the Hot Plate; occasionally a diatom will jump out of position if, I think, the temperature is a bit too high. Good, they came through that, and now for the next hurdle, the clearing in Xylol; apart from getting dislodged one will sometimes not clear, but still all well. Now the Canada Balsam on and O.K. Just a tiny drop to cover the diatoms and I let it stand cold for 40 hours.

Now for the moment of truth, the putting on of the cover glass and the setting of the guide ring right. Cheers,

I've made it! Imagine one's feelings of relief and joy when one sees one's effort · crowned with success.

If anyone thinks there is no fun in mounting diatoms, try doing a multi-mount. It really is a most fascinating and thrilling game and one which I thoroughly enjoy.

When making up this box I wondered whether to send this mount or a smaller one of say 40 or 50, but there is not much point in doing these things unless one can show them to others, and this is not the only one. So I decided to chance it. It will be interesting to see how it stands up to the travel without any disarrangement. If any of the diatoms do come adrift please notify Mr. Darby; he will advise me and I will try to replace it with ·something similar.

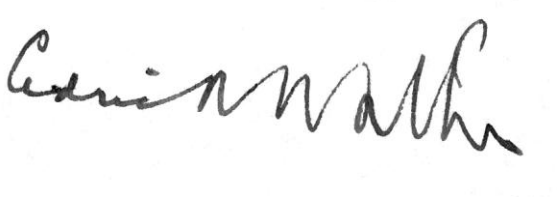
The adhesive was Glycerin Albumen.

CONCLUSION

Well, Gentlemen, that is the end of my story of "Operation Diatoms" and how I have carried it out. Others may have different methods of doing these things, but that is so in other forms of microscopical mounting. We all have our pet ways; the principle thing is whether they achieve the desired results.

And now, where do we go from here? Having progressed thus far in mounting; I can now turn my attention to the more studious aspects of Diatoms.

First of all, comes the continual question, "What is it?" They have to be identified; this is another challenge and some method has to be worked out, so D.V. I hope to deal with that subject in my next Diatom contribution.

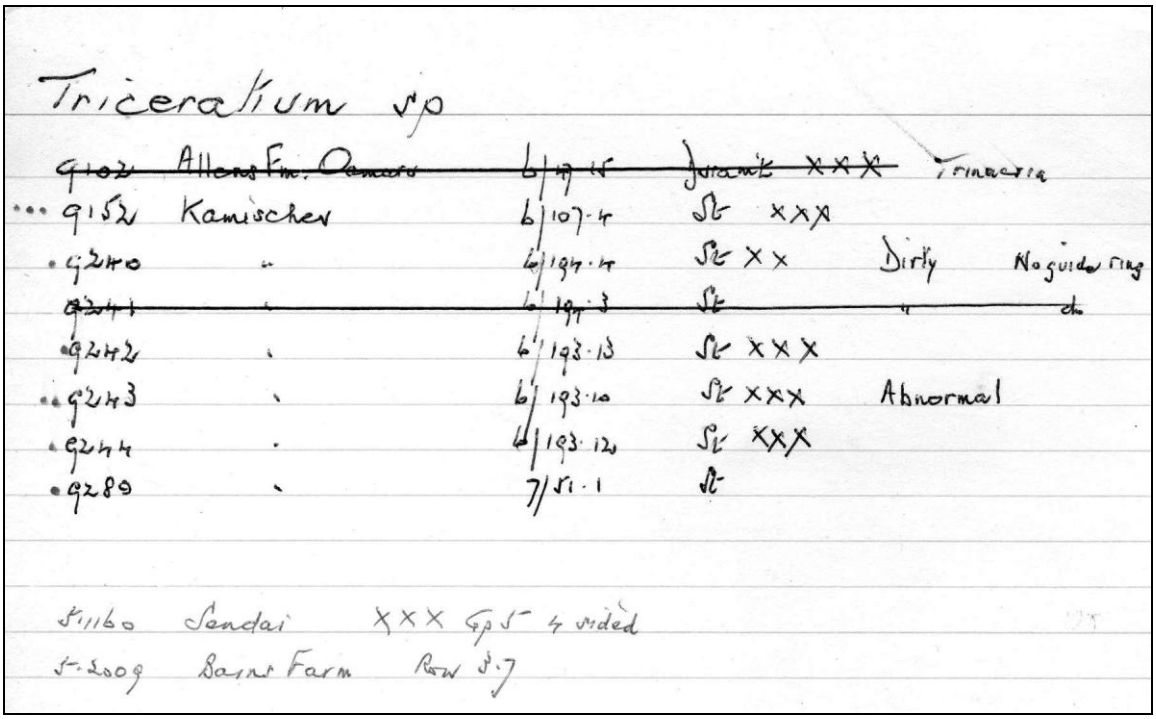


P.S. In one of the notebooks recently, a member wrote that he envied those who do mounting, but he was 62 and felt he was too old!

That "young fellow" can take heart and make a start. He'll never regret it. I did not commence serious mounting in any form until I started to retire, when I was 70 - and that was getting on for five years ago. We are never too old to learn.

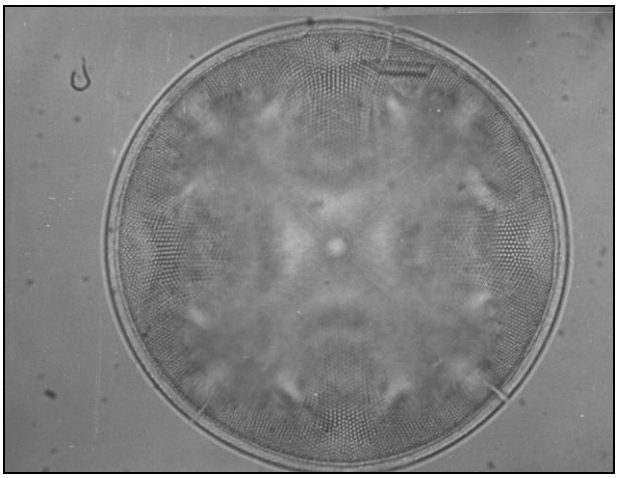
APPENDIX B

Cedric Norman Walter's Slide Index

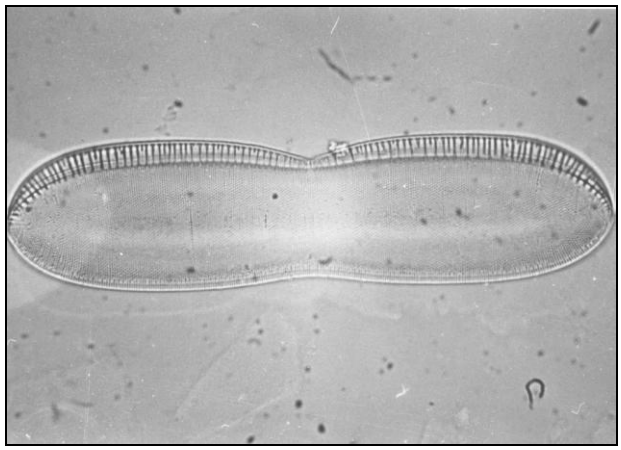


Sample Index Card

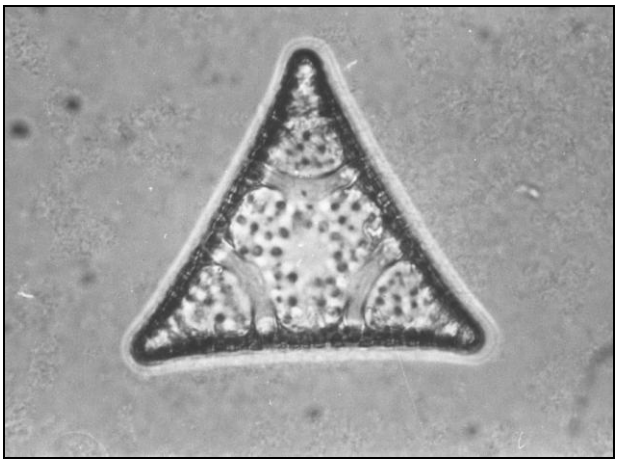
The Card Index also contained a number of photographs. It may be that these were taken by A.V. Dodge, who had custody of the index and slide collection for a number of years.



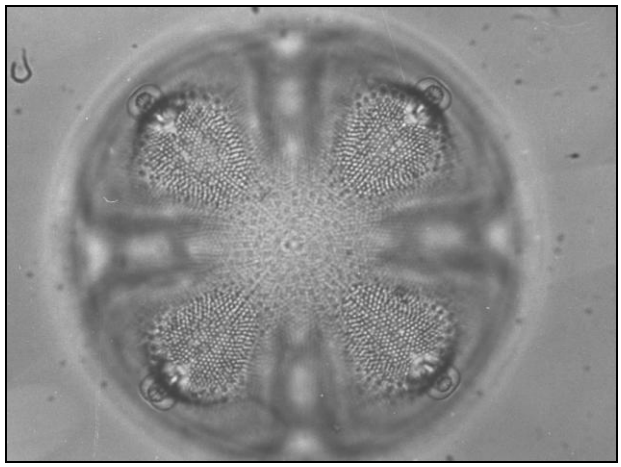
Caption: 9163



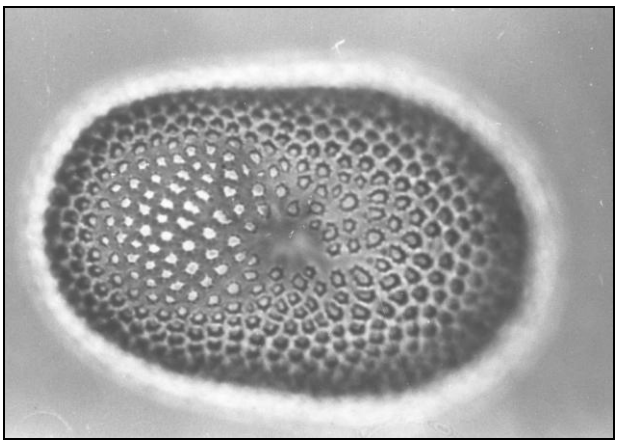
No Caption



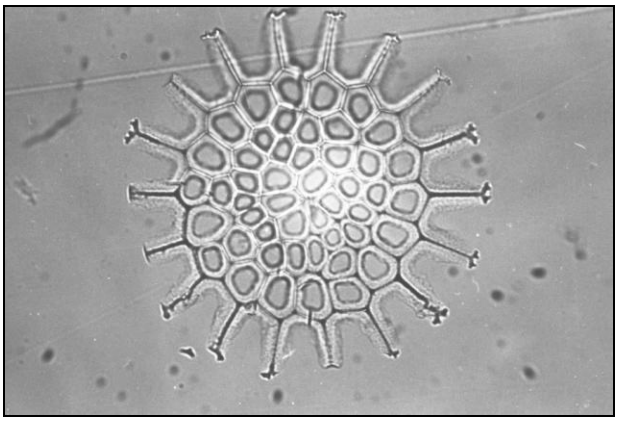
Caption: 9151 *Triceratium fenestratum*. Styrax. 4mm. Kpl8. 8”



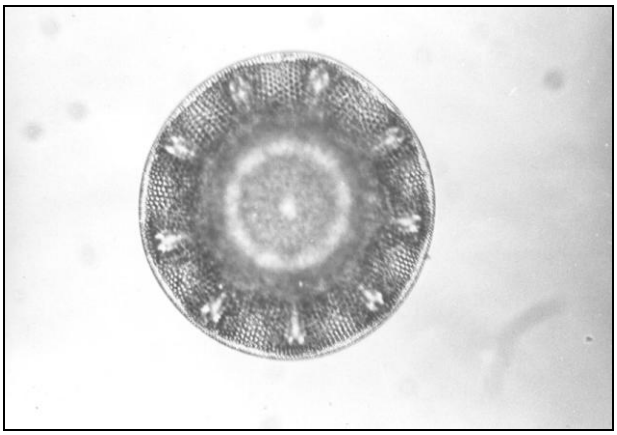
Caption: *Aulacodiscus petersii* Ehr. 4mm. Kpl8. 7”



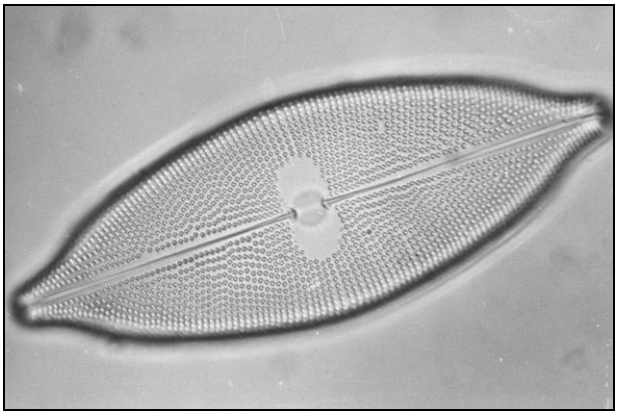
Caption: 9153. 4mm. Kpl8. 8½". Breen's, Oamaru N.Z.



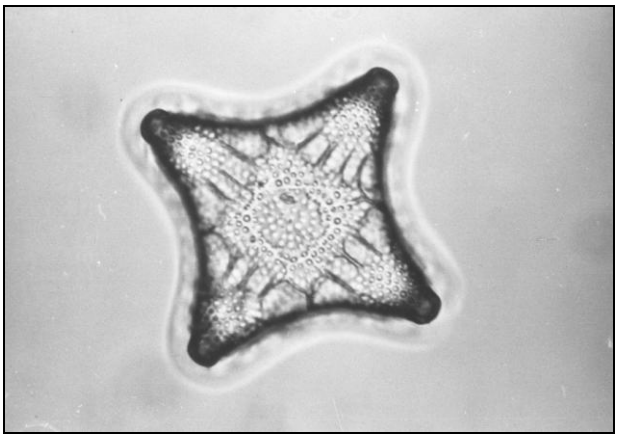
Caption: 9154. 4mm. Kpl8. 7". Breens, Oamaru



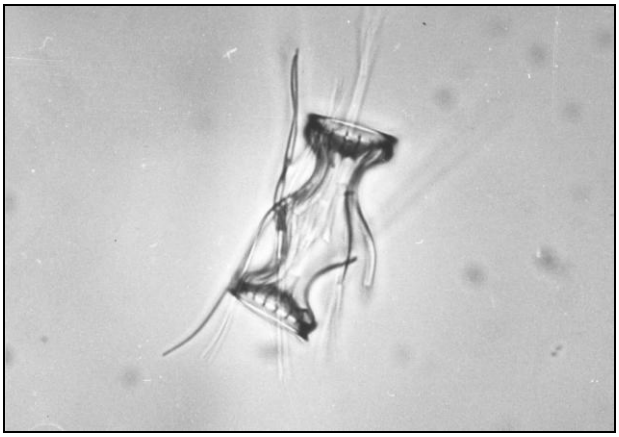
Caption: 9155. *Aulacodiscus angulatus* Grev. 4mm. Kpl8. 7"



Caption: 166



Caption: 8



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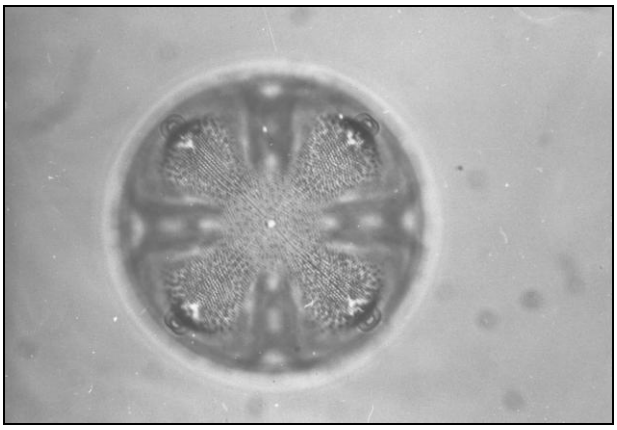

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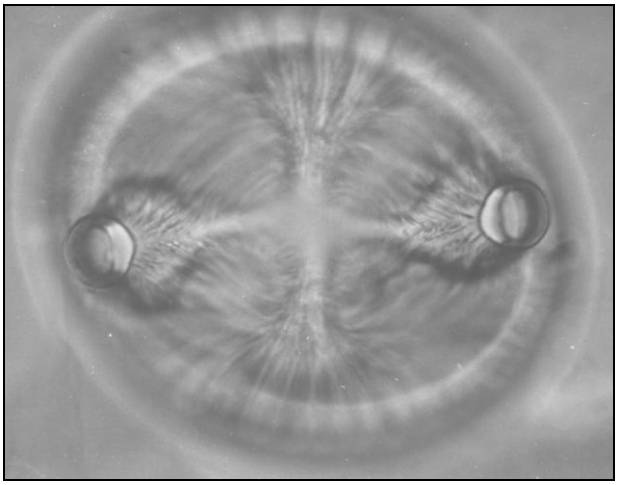

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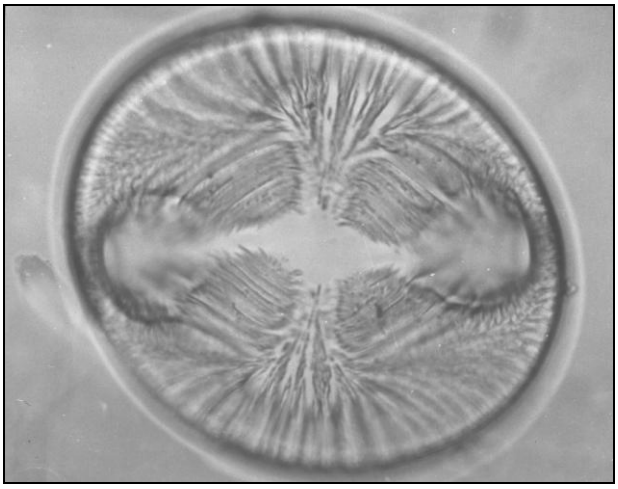

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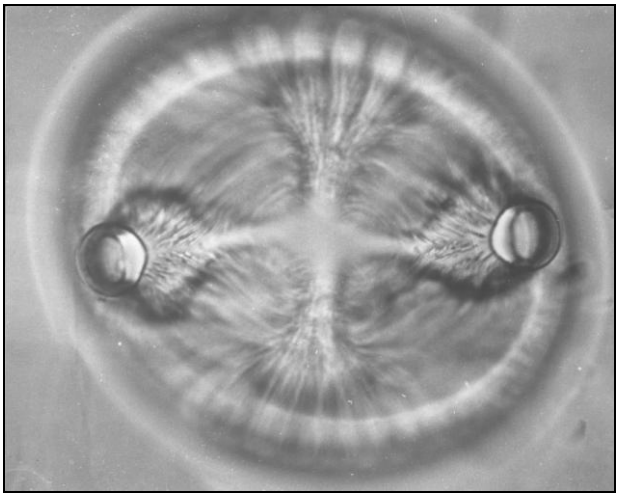

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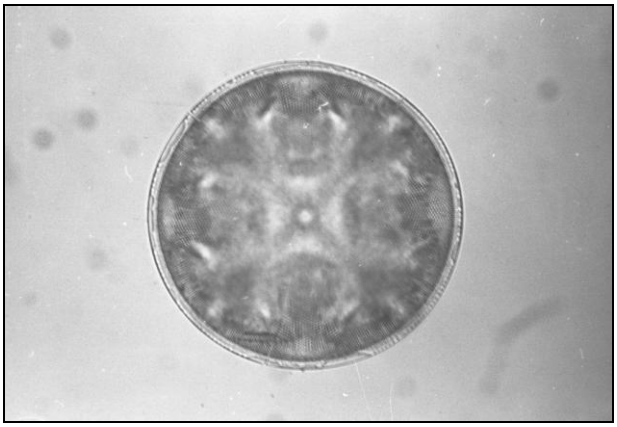

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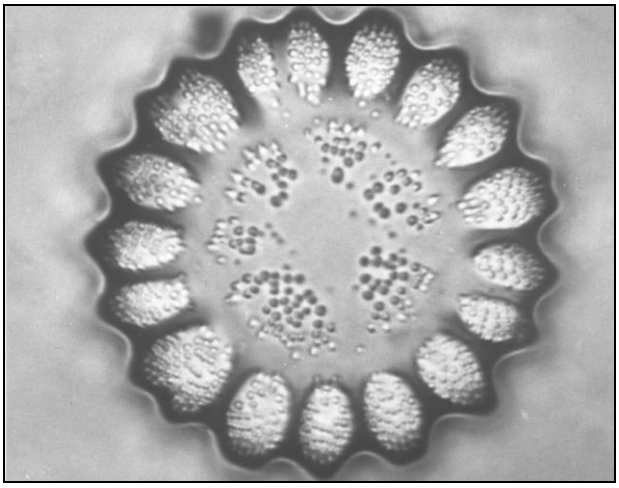


No Caption

A tray of C. N. Walter mounts

APPENDIX C

Samuel Walter (1600-?)
m. ?
Jeffrey Walter (1625-?)
m. Mary ?
Richard Walter
Elizabeth Walter (1652-?)
Samuel Walter (1654-?)
Thomas Walter (1657-1754)
m. Susanna Parson (1661-1706)
Thomas Walter (1685-?)
William Walter (1690-?)
Ann Walter (1692-?)
Henry Walter (1700-1742)
m. Mary ?
Sarah Walter (1734-?)
Ann Walter (1737-?)
m. Elizabeth Fox
Mary Walter
Thomas Walter (1725-?)
Henry Walter (1727-1806)
m. Mary Godfrey (1734-?)
Thomas Walter (1755-1840)
Henry Walter (1757-1845)
m. Ann Durant (1763-1838)
Henry Walter (1786-1861)
m. Ann Booth (1788-1874)
Henry Thomas Walter (1816-?)
Ann Walter (1819-?)
Thomas Walter (1819-1908)
m. Esther Kelly (1827-1876)
William Norman Walter (1856-1938)
Thomas Norman Walter (1857-1935)
In the 1891 Census @ 159 Huddlestone Road, Islington:

(the home of Ellen's widowed mother - Sophia D. Walter
Thomas Walter, aged 33, an Actor, b. Islington
In the 1901 Census @ 11 Union Road, Islington
(the home of Ellen's widowed mother - Sophia D. Walter
Thomas Walter, aged 43, a Theatrical Stage Manager,
b. Islington, London
In the 1911 Census @ 3 Ivy Lane, Brockley, London, S.E.:
Thomas Walter, aged 53, a Theatrical Manager, b. Islington, London

m. Ellen Walter (1861-1941)
BMD Marriage June Quarter 1889 Pancras Vol.1b. pg.153.
In the 1891 Census @ 159 Huddlestone Road, Islington:
(the home of Ellen's widowed mother - Sophia D. Walter
Ellen Walter, aged 29, an Actress, b. St. Giles, London
In the 1901 Census @ 11 Union Road, Islington
(the home of Ellen's widowed mother - Sophia D. Walter
Ellen Walter, aged 39, b. Bloomsbury, London
In the 1911 Census @ 3 Ivy Lane, Brockley, London, S.E.:
Ellen Walter, aged 29, b. Bloomsbury, London

├──Cedric Norman Walter (1890-1973)
b. 23rd November 1890, Islington, Middlesex
BMD Birth March Quarter 1891 Islington Vol.1b. pg.198.
In the 1891 Census @ 159 Huddlestone Road, Islington:
(the home of Ellen's widowed mother - Sophia D. Walter
Cedric N. Walter, aged 4 months, b. Islington
In the 1901 Census @ 11 Union Road, Islington
(the home of Ellen's widowed mother - Sophia D. Walter
Cedric N. Walter, aged 10, b. Islington
In the 1911 Census @ 3 Ivy Lane, Brockley, London, S.E.:
Cedric Norman Walter, aged 20, a Chartered Accountant's
Clerk, b. Islington, London
Elected to the Quekett Microscopical Club 26th May 1956
In the 1962 Q.M.C. Membership List:
Walter, C. N., 32 Stanley Avenue, Beckenham, Kent
In the 1969 Q.M.C. Membership List:
Walter, C.N., F.R.M.S., Rotherwood, 32 Stanley Avenue,
Beckenham, Kent, BR3 2PX
d. 27th October 1973 Bromley, Kent
BMD Death, aged 83, December 1973 Bromley Vol.5a. pg.941.

m. Violet Kingsford
BMD Marriage September Quarter 1914 Lewisham
Vol.1d. pg.2300.

├──Daisy E. Walter (1919-?)

└──Patrick Victor Walter (1925-2004)

├──Hubert Norman Walter (1893-1918)
In the 1901 Census @ 11 Union Road, Islington
(the home of Ellen's widowed mother - Sophia D. Walter
Hubert N. Walter, aged 8, b. Islington, London

├──Ellen Adeline Walter (1899-1988)
In the 1901 Census @ 11 Union Road, Islington
(the home of Ellen's widowed mother - Sophia D. Walter
Ellen A Walter, aged 1, b. Islington, London
In the 1911 Census @ 3 Ivy Lane, Brockley, London, S.E.:
Ellen Adeline Walter, aged 11, at School, b. Islington, London

└──Albert Norman Walter (1903-1974)

In the 1911 Census @ 3 Ivy Lane, Brockley, London, S.E.:
Albert Norman Walter, aged 8, at School, b. Islington, London

- William Walter (1821-1826)
- Mary Walter (1827-1901)
- Charles Edward Walter (1831-1906)
- John Walter (1788-1826)
- William Walter (1792-1795)
- Elizabeth Walter (1795-1851)
- Esther Walter (1800-?)
- Stephen Walter (1802-?)
- Mary Walter (1807-1832)
- Alexander Walter (1759-?)
- Michael Walter (1761-?)
- Elizabeth Walter (1764-?)
- John Walter (1767-?)
- Esther Walter (1770-?)
- Mary Walter (1772-?)
- William Walter (1775-1847)
- Sarah Walter (1777-?)
- Harriot Walter (1782-?)
- John Walter (1728-?)
- Esther Walter (1732-?)
- Francis Walter (1702-?)
- Benjamin Walter (1706-?)
- Jeffrey Walter (1662-?)
- John Walter (1665-?)
- Martha Walter (1668-?)

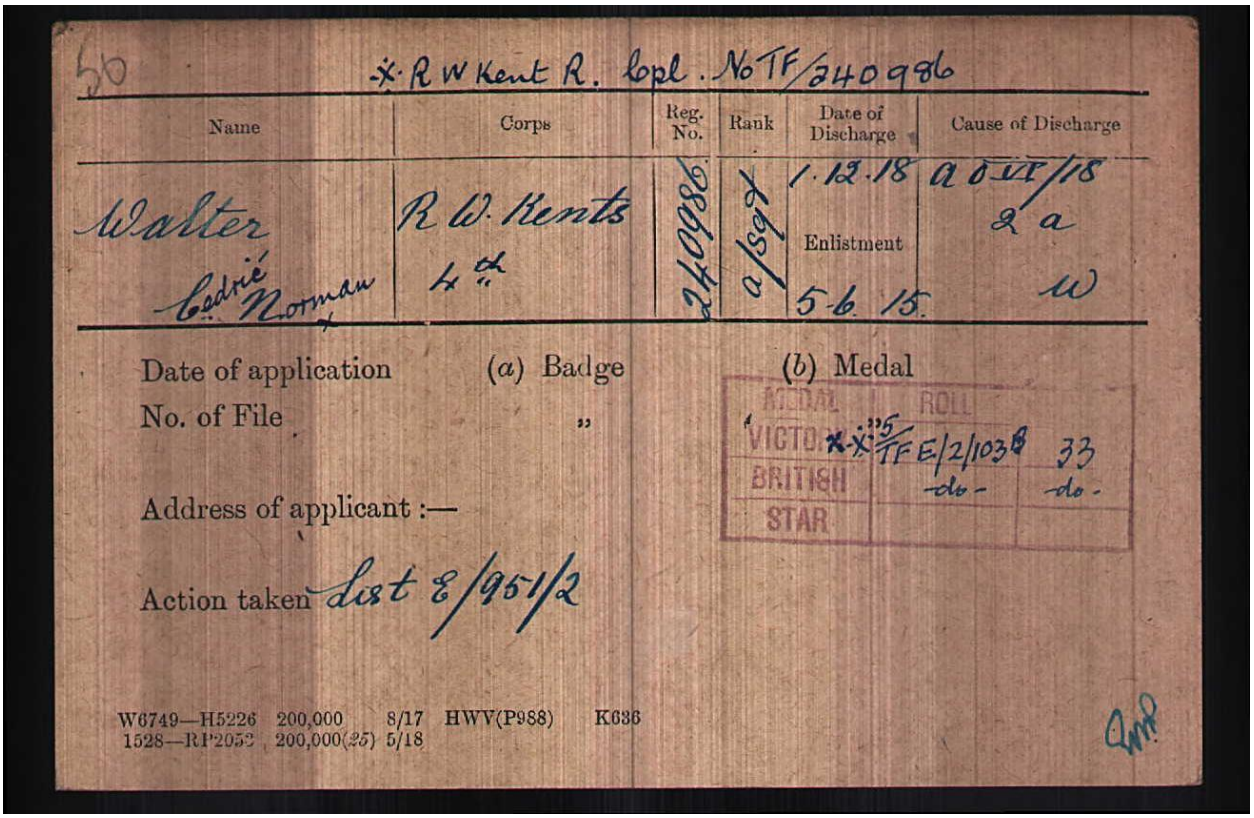
GELATINEX Limited.
The Companies Act, 1929.

AT an Extraordinary General Meeting of the Members of the above named Company, duly convened, and held on the 4th day of September, 1935, the following Extraordinary Resolution was duly passed:—

"That it has been proved to the satisfaction of this Meeting that the Company cannot, by reason of its liabilities, continue its business, and that it is advisable to wind up the same, and accordingly that the Company be wound up voluntarily; and that Cedric Norman Walter, Incorporated Accountant, of 290, Finsbury Pavement House, London, E.C.2, be and he is hereby appointed Liquidator for the purposes of such winding-up."

(099) THOS. G. S. BIDWELL, Chairman.

The London Gazette 13th September 1935


50

×·R W Kent R. Lpl. No TF/240986

Name	Corps	Reg. No.	Rank	Date of Discharge	Cause of Discharge
Walter Cedric Norman	R W Kents 4th	240986	a/Sgt	1.12.18 Enlistment 5.6.15.	a 6.VI/18 2a W

Date of application (a) Badge (b) Medal

No. of File "

Address of applicant :—

Action taken List 8/951/2

MEDAL ROLL
VICTORY — ×·×·5/TF E/2/1038 — 33
BRITISH — do. — do.
STAR

W6749—H5226 200,000 8/17 HWV(P988) K636
1528—RP2052 200,000(25) 5/18

Medal Roll Index Card 1914-1920

Cedric Norman Walter

(23rd November 1890 - 27th October 1973)