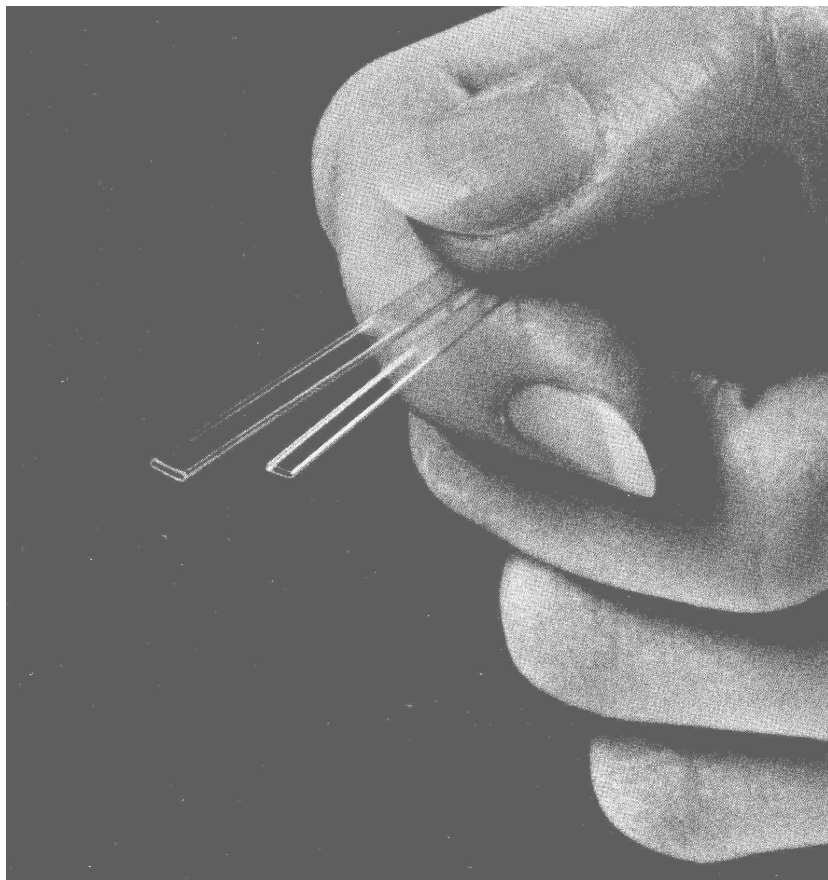


VITROTUBES

**techniques and experiments
by Prof. John O. Harris**

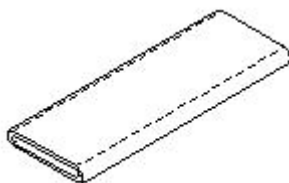
**RECTANGULAR GLASS CAPILLARIES FOR MICROSCOPIC EXAMINATION
OF BIOLOGICAL MATERIALS ESPECIALLY PREPARED FOR SCHOOLS
AND RESEARCH LABORATORIES.**



**VitroCom
8 Morris Avenue
Mountain Lakes, NJ 07046**

TABLE OF CONTENTS

Indroductio.....	2
Handling and Mounting Vitrotubes	3
I. Observation of Diatoms, Algae, and Other Microorganisms in Natural Aquatic Enviroment.....	4
II. Study of the Biology of the Water-mud Interface in Natural Environments	5
III. Examination of Soil Micro-organisms	6
IV. Preditory Bacterial Forms.....	7
V. Suggestions for Fixing – Staining – Etc.....	9
VI. Vitrotubes in Standard Laboratory Procedures	10



Originally published by Vitro Dynamics in 1975

Vitro Dynamics name changed to VitroCom in 1995

INTRODUCTION

There are two significant advantages to the use of Vitrotubes in biological investigations. First, the capillaries furnish an environment more nearly like that of soil or mud in nature, than any method possible till now. Thus the critical spacial relations with the attendant physical-chemical environmental determining factors can be met within the tubes. By placing the capillaries at various locations in nature (or simulated environments in the laboratory) the natural gradients of oxygen, carbon dioxide (and other gases), the range of oxidation-reduction potentials, pH, and nutrient levels can be established within the capillary allowing the selective effects of a specialized environment to be met.

The second advantage has to do with the rectangular dimensions of the capillary cells. The flat surfaces (optically precise) allow the interior contents of the tube to be observed under the various magnifications available with the compound microscope. Thus the development and growth of various organisms can be studied in an undisturbed situation. This is quite important for fragile attached forms which could not be observed in any other manner.

The Russian biologists have done practically all of the research to the present time using this type of capillary. They perfected the glass-making process a number of years ago and have gone to elaborate and complicated types of capillary equipment. *At this point it should be emphasized that the essentials of capillary size and rectangular cross-section are met by Vitrotubes.*

A large volume by V. Perfilev and D.R. Gabe titled "Capillary Methods of Investigating Microorganisms" was published in Moscow in 1961. An English translation was made available by the University of Toronto Press in 1969. This book gives details of the capillary making process, discusses specialized designs for various uses, and describes microorganisms they have observed in soil and lake bottom sediments.

Editor's note: Vitrotubes described in this manual are made of heat and chemical resistant expansion 3.3 Borosilicate glass. The path lengths are held within 10% of given size. Commercially available stock sizes of Vitrotubes can be found on the VidroCom.com website.

HANDLING AND MOUNTING VITROTUBES

While the Vitrotubes have sufficient strength to be handled individually, most persons will find it advantageous to mount them on an ordinary glass microscope slide. An epoxy or silicone rubber cement is probably the best method to give a permanent stable mount. However paraffin wax or many types of glue or other adhesive combinations can be used for temporary mounts. In many biological experiments it is well to use the whole range of capillary sizes on a single slide particularly in preliminary studies to determine the optimum dimensions for the particular study. The Vitrotubes should be mounted flat against the surface and parallel with the long dimensions of the slide, Figure 1.

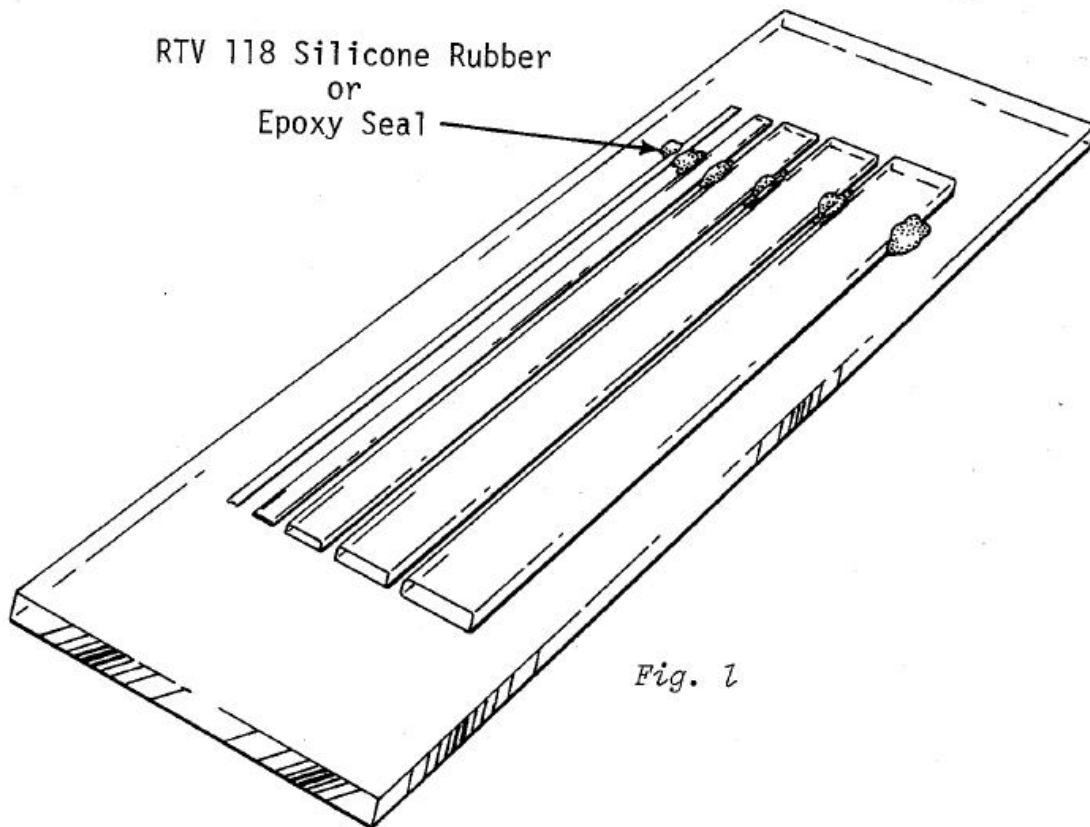


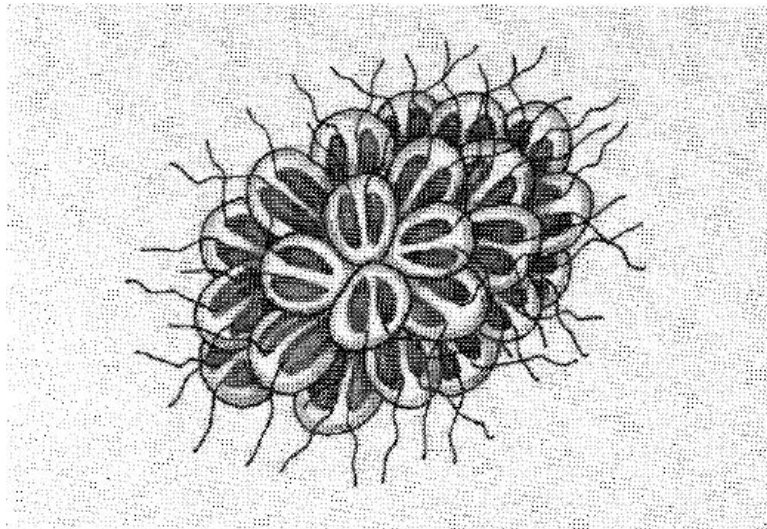
Fig. 1

Sealing at a single point between the Vitrotubes and an ordinary glass slide will be sufficient for most uses. Because most compound microscopes have mechanical stages for holding and moving glass microscope slides, the mounted preparation requires no special adaptations for microscopic observation.

I. OBSERVATION OF DIATOMS, ALGAE, AND OTHER MICROORGANISMS IN NATURAL AQUATIC ENVIRONMENT

1. Arrange slides with Vitrotubes attached in various locations in natural water systems such as springs, streams, and lakes. Plan the distribution so the slides will not be disturbed by water flow or water level fluctuations.

2. After five days colonies of algae and diatoms can be found inside the capillaries; obviously the longer the slides remain in the natural situation, the more complex and the greater will be the populations within the tubes.



*Fig. 2 SYNURA UVELLA – magnified 500X –
odor producing algae in potable water supply.*

3. Following removal from the water, the bottom side of the slide should be cleaned with lens tissue as well as the uppermost flat surface of the Vitrotube. Careful!

4. If the microscopic examination is done within a few hours it is not necessary to seal the ends of the tubes. Melted paraffin wax applied to the ends gives a satisfactory seal holding for several weeks. For a permanent seal the tips can be melted and sealed or a permanent type of cement can be used.

5. It is on microscopic examination that the real value of the Vitrotubes becomes most apparent. One can examine the living forms as they have developed and in a living undisturbed condition. As with all microscopic procedures care must be given to having proper illumination to match the magnification. Colored organisms such as the photosynthetic algae with their chlorophyll or diatoms with the refractile silica shells can be easily observed but protozoa and bacteria may need reduced light.

6. For high power magnification (1000X) with oil immersion lenses, apply the immersion oil generously to the capillary so it not only fills the space between the lens and the upper surface of the capillary but also forms a continuous layer between the *bottom* of the capillary rectangle and the supporting microscope slide.

7. Where available, use of the phase contrast microscope is the best technique available for study of living cells. All five sizes of the rectangular capillaries can be used under phase contrast microscopes.

II. STUDY OF THE BIOLOGY OF THE WATER-MUD INTERFACE IN NATURAL ENVIRONMENTS

1. Because of the difficulty of studying of this region without severe disturbance little is known of the biological community in sediments of streams, lakes, and reservoirs.

2. Supported Vitrotubes can be placed in an upright position at various depths in the bottom sediments. After time for the capillaries to become colonized (5 days to 5 weeks) they can be removed to the laboratory for microscopic study.

An alternate method is to collect some mud with the overlaying water in pint jars or beakers and bring these into the laboratory where temperature and evaporation can be controlled. By simply placing the capillaries on their supporting glass upright in the sediment as it settles, one reproduces within the capillaries the environment as it comes to equilibrium. In this procedure one should have about one third of the capillary in the water and two thirds

in the sediment phase. Most of the particulate matter will not penetrate into the capillaries.

3. Microscopic examination will reveal a diverse community of many types of protozoa, bacteria, and some algal forms. Because they have not been disturbed, attached protozoan forms such as *Vorticella* are readily found. It is with such preparations that the Russians have found the numerous odd bacterial predatory colonies which are discussed later.

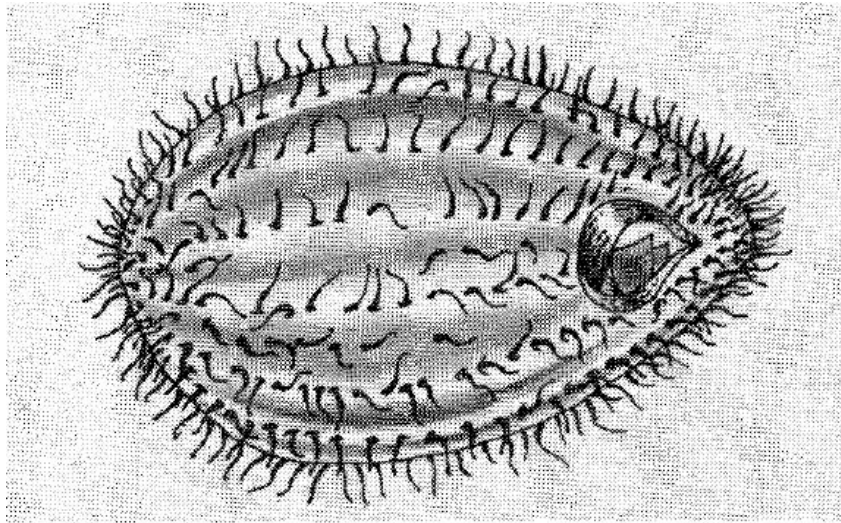


Fig. 3 CILIATE PROTOZOON – visible in Vitrotubes with reduced lighting.

4. Because these preparations will remain active for several days even when sealed at the ends, one can observe growth of filaments, feeding habits of predators and other changes as they occur.

III. EXAMINATION OF SOIL MICROORGANISMS

1. Placing the Vitrotubes in soil requires more care than when used in water or mud. The Russians have devised a metal holder with the capillaries sandwiched between two sheets of metal and one end only exposed. Such an assembly can be used for soils in the field but it is relatively easy to bury the mounted capillaries in containers of soil for controlled laboratory studies.

2. Selective action of various nutrients can be demonstrated by coating the inside of the capillaries with the desired food substance. For example 5% starch, melted butterfat, or milk passed through the tube prior to placing in the soil will leave a thin coating within the tube which will selectively enrich for starch, fat, or protein digesting types of soil microorganisms.

3. As in aqueous environments, about a week is needed for the colonization of the tubes. Care should be used to *remove* all soil particles from the slides before placing on a microscope to prevent the possibility of damage to the microscope lens.

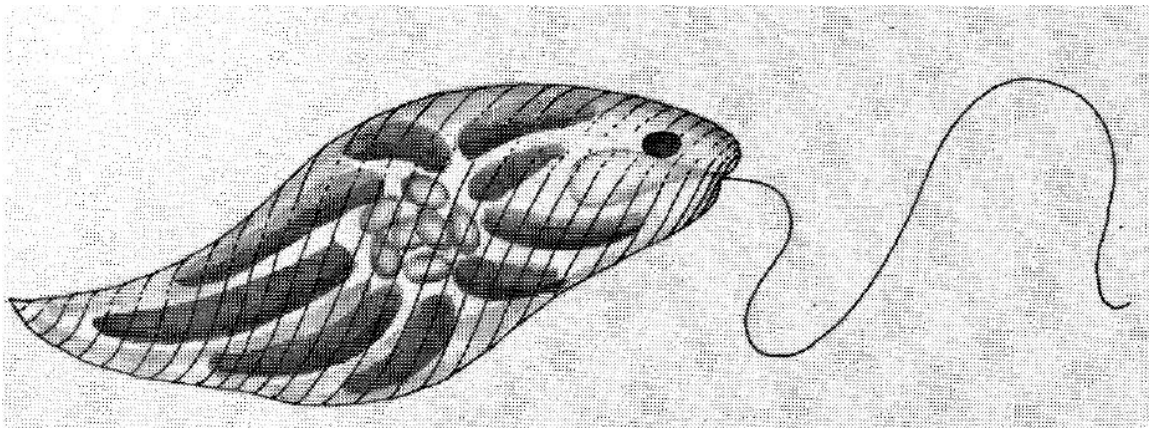


Fig. 4 EUGLENA VIRIDIS – 1000X magnification – polluted water algae.

4. Predominant among the biological types seen in most soil studies will be the streptomyces and rod-like bacteria growing on whorls and spirals as they do in nature. Protozoa of various kinds, as well as filamentous fungi are commonly observed.

IV. PREDTORY BACTERIAL FORMS

1. The Russian microbiologists have observed and described a number of predatory types of soil and water bacteria. These are unfamiliar to most biologists since they act in a very fragile colony-like growth which cannot be disturbed and still be observed. The rectangular capillary has changed the situation and now this group can be studied in a living state and their role in nature studied.

2. The typical action of the bacterial predator is for the individual bacterial cells to associate together in some form of organized colony which acts to trap, hold, surround, and eventually digest the prey. Protozoa, algae, fungi, and other bacteria may serve as food for the predators. Most of the predatory types can grow on dead organic matter or soluble nutrients as well as living prey.

3. Following are brief descriptions of some predatory bacterial types. For details consult the Russian publications.

a. *Dictyobacter* species – net-like formation forming digestive sacs and commonly trapping "iron" bacteria.

b. *Cyclobacter* species – rod-like bacterial predators, threads of cells form terminal loops with a lasso like action - figure eight shapes common.

c. *Trigonobacter* species – network of their rods – often form triangular mesh because of three cell associations.

d. *Streptobacter* species – complex mobile cobweb formation producing much sticky mucous.

e. *Teratobacter* species – a complex colonial form of thousands of cells forming loops and outgrowths which act as prehensile and adsorptive "organs".

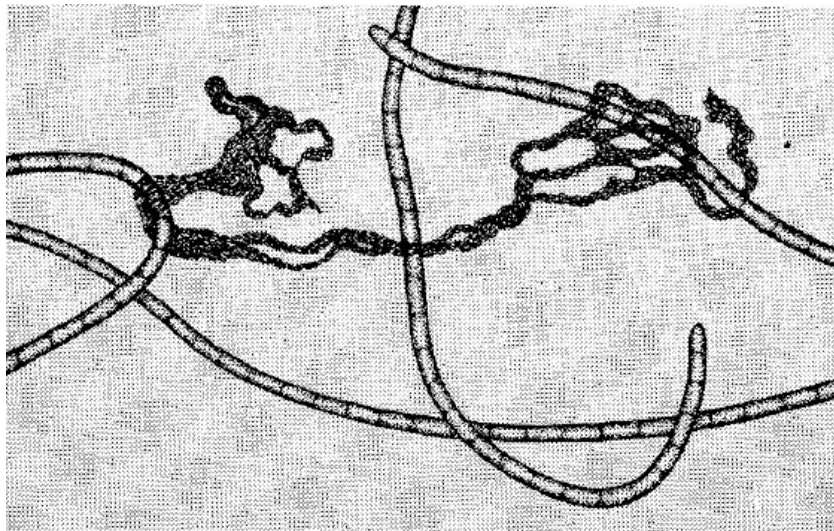


Fig. 5 TERATOBACTER ATTACKING BEGGIATOA – as viewed in rectangle glass capillaries by Russian biologists.

V. SUGGESTIONS FOR FIXING – STAINING – ETC.

1. Because the microbial colonies grow adherent to the inner capillary walls it is possible to kill them, stain them, and otherwise manipulate the cells and colonies as in other micro-techniques.

2. For killing and fixing – formalin (1%) is probably the most useful although other disinfectant-fixative solutions may be used.

3. Vital stains such as methylene blue can be used for study of living cells. For killed and fixed preparations the choice of stain will be dictated by the purpose of the study.

4. In general two procedures may be used to introduce solutions such as fixatives or stains into the capillaries. First the mounted Vitrotubes could be dipped into the desired solution in a glass or beaker. This requires considerable volume and also further cleaning of the glass surfaces. The second procedure calls for placing the capillaries at about a 45 degree angle, adding the solution slowly and carefully at the top opening with an eye dropper or Pasteur pipette and allowing the liquid to flow through the capillary. Blotting paper judiciously applied to the lower end will speed flow through the tubes.

5. Electron microscope research workers who have examined the Vitrotubes suggest it should be quite easy to fix, and embed in plastics the colonial forms inside the capillaries. The glass could be broken away and the embedded biological forms sectioned and prepared for electron microscopy.

6. It is apparent that the use of these tubes awaits only the ingenuity of the experimenter in adapting them to standard procedures.

VI. VITROTUBES IN STANDARD LABORATORY PROCEDURES

A. AS COUNTING CHAMBERS:

1. The Russians have published statistical data showing the validity of using the rectangular capillaries as counting chambers for counting microorganisms in water, milk, food, and other environments.

2. The internal volume can be determined by (a) volumetric titration with a standard alkali-acid methods, (b) calculation from measurements of the physical dimension using a stage micrometer and calibrated depth measurement on the microscope.

3. For ease and time considerations, the suspension to be counted should be diluted or concentrated so that the total per tube is in the range of 100 to 500 cells. The size of the capillary that works best depends on the magnification. With the high magnification of the oil immersion lens, the .05mm ID tube should be used.

B. FOR EXAMINATION OF MOTILITY:

The capillary tubes can readily substitute for the usual hanging-drop or well-slide in study of moving microorganisms. They can be easily filled by capillarity and once prepared (especially if the ends are sealed) the

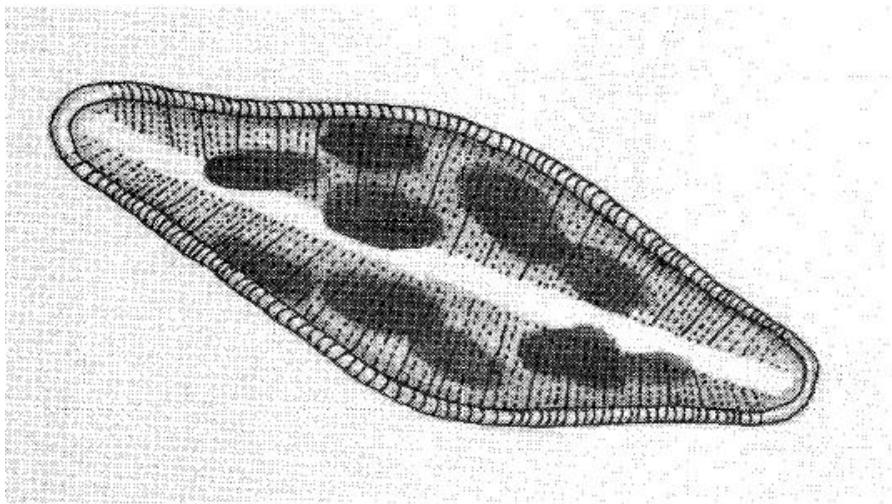


Fig. 6 DIATOMA VULGARE- magnified 1500X - found in sand filters of 'Water treatment plant.

internal fluid is not subject to the physical movement that often plagues the hanging-drop observer.

C. CHEMOTAXIS:

Gradients of various beneficial or harmful chemicals can readily be prepared and movement of living cells toward or away from the compound observed.

D. PHOTOTOXIS:

A point of light focused on the bio-community within the capillary while under the microscope enables observation of any movement toward or away from the light. These studies should be done in a darkened room.

E. SINGLE – CELL ISOLATION:

The Russians have devised elaborate equipment with the capillary tube as the key component for isolation of single cells of microorganisms. A micromanipulator is necessary.

IN CONCLUSION

In this little manual, suggestions have been given for the use of Vitrotubes in several types of biological applications. Certainly in the areas of physical science, these rectangular capillaries will also find many uses. Melting point determination, micro-fusion procedures, precipitate formation, and diffusion rate studies come immediately to mind as methods in which the microscope is often used to add precision to measurement.

Vitrotubes value to you will depend upon your own inventiveness and ability to put the fine optical characteristics and precision of manufacture to its ultimate applications.

SELECTED REFERENCES

1. Capillary Methods in Investigating Microorganisms. B.V. Perfilov and D. G. Gabe. University of Toronto Press. 1969.
2. Algae of the Western Great Lakes. G. W. Prescott. W. C. Brown. 1962.
3. A Manual of the Fresh Water Algae in North Carolina. L. A. Whitford and G. J. Schumaker. North Carolina Agric. Expt. Sta. Tech. Bul. No. 188. 1969.
4. Protozoology. R. R. Kudo. Chas. C. Thomas. 1966.
5. Marine Microbial Ecology. E. J. F. Wool. Reinhold. 1969.

LABORATORY SHOP NOTES

ATTACHING SMALL RUBBER OR PLASTIC TUBING:

Small diameter plastic, rubber or glass tubing can be joined to Vitrotubes with silicone rubber adhesive (RTV 118). Two millimeters in from the open end of the Vitrotube apply a thin ring of liquid rubber. Slip the round tubing over the silicone rubber ring and allow to set for 12 hours before use.

Additional silicone rubber can be added to the end of the round tube with a pin or paper clip to assure a liquid tight seal. Vitrotubes 100 mm long are available and will be flat on a microscope stage or standard slide when round tubing is attached.

CUTTING:

Cutting Vitrotubes to shorter lengths is accomplished by abrading a line on the flat surface. A fine abrasive stone or jeweler's file is suitable for scoring a line. A slight bending pressure away from the scored line will snap the Vitrotube.

IDENTIFICATION OR MARKING VITROTUBES:

Use dots of colored lacquers (or assorted color marking pens) each color denoting a different digit, a system used with electrical resistors. The color coded number indicators on the Vitrotubes are referenced in a notebook with corresponding number and details of contents. Assorted colored pens with felt tips that will mark glass are available at stationery stores.

HOLDING VITROTUBES:

Small sizes of Vitrotubes are conveniently held on the microscope stage by placing them on a standard 3/4" x 1" slide. The Vitrotubes are secured to the standard slide with a dot of adhesive (glue, wax or cement) near one end of the Vitrotube. A dot of transparent household cement (such as "Duco") is applied with a toothpick, straight pin or fine wire.

TELESCOPING VITROTUBES:

Telescoping small vitrotubes into larger sizes will increase the range of experiments that can be performed using rectangle capillaries. Single cell isolation, observing chemical reactions, introducing predator type micro-organisms and constructing micro-cuvettes are a few examples. The .05 path length Vitrotube (#5005) will slide into a .2 path length Vitrotube (#3520). A 0.1 mm I.D. (#5010) fits into a .4mm I.D. (#2540). See vitrocom.com for the complete listing Vitrotubes, for other telescoping combinations.

OTHER APPLICATIONS:

Vitrotubes are suitable for use in Micro Spectrophotometer in the visible range, also in the fluorescent photometer. Fused silica Vitrotubes for the ultra violet range are also available.

Vitrotubes 4mm long are suitable for implanting or infusion of known quantities of microorganisms into other solutions or media.

