# A METHOD FOR CYTOLOGICAL INVESTIGATION OF ALGAE

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The pollen tube technique used for cytological studies of the microgametophyte of Angiosperms was adapted for cytological and morphological studies of algae. This method mainly follows that outlined by Eigsti (1940) for the procedures used in cultivating pollen tubes and the staining and permanent mount technique used in cytological investigations (Eigsti, 1942).

The many hours and many steps necessary in present day methods have stimulated this technique for fast permanent mounts requiring a rather simple procedure. In classes studying morphology and cytology of algae, time would not allow a consideration of personal investigation by the student. It is with two objectives that this report is given. (1) To introduce a new method for detailed cytological investigation of algae on the part of experienced technicians, and (2) to point out to teachers of cytology that this method can easily be introduced as a means of personal investigation by the student.

Vigorous stocks of algae provide the best material. When nuclear phases are desired, examination at various intervals throughout a 24-hour day can be used to acquire proper stages of cells in division.

The desired quantity of the alga should be transferred to a glass slide with a minimum amount of water or fixative. Before the alga has any opportunity to dry out, a thin film of agar imbedding solution is added to the alga mount on the slide. The agar must be liquid at the time of application to the slide and all arrangement of the alga in the agar must be accomplished before the agar solidifies. Only an amount necessary to cover the alga is desired.

The imbedding solution consists of 1 gram of agar and 5 grams of sucrose completely dissolved by boiling in 100 ml of distilled water. The agar mixture is applied to the slide at a temperature of about 40° C. Any agar remaining can be sterilized and kept for subsequent use. A constant temperature water bath for the agar facilitates the operations at the time the mounts are prepared.

As soon as the agar and alga are on the slide, allow the film to dry. Drying is necessary to prevent the film from loosening from the slide as the slide is immersed in reagents used to stain and dehydrate the material. The amount of drying is best judged from experience and is dependent upon the species of alga used. Certain gelatinous forms can withstand drying for several hours. As a general rule, however, the drying process is accomplished within 10 or 15 minutes.

The staining series is made up as follows:

1. *Aceto-carmine*

The aceto-carmine stain is made by reflux condensing for 5 hours at a slow rate one teaspoon of carmine to 100 cc of 45% acetic acid. It is

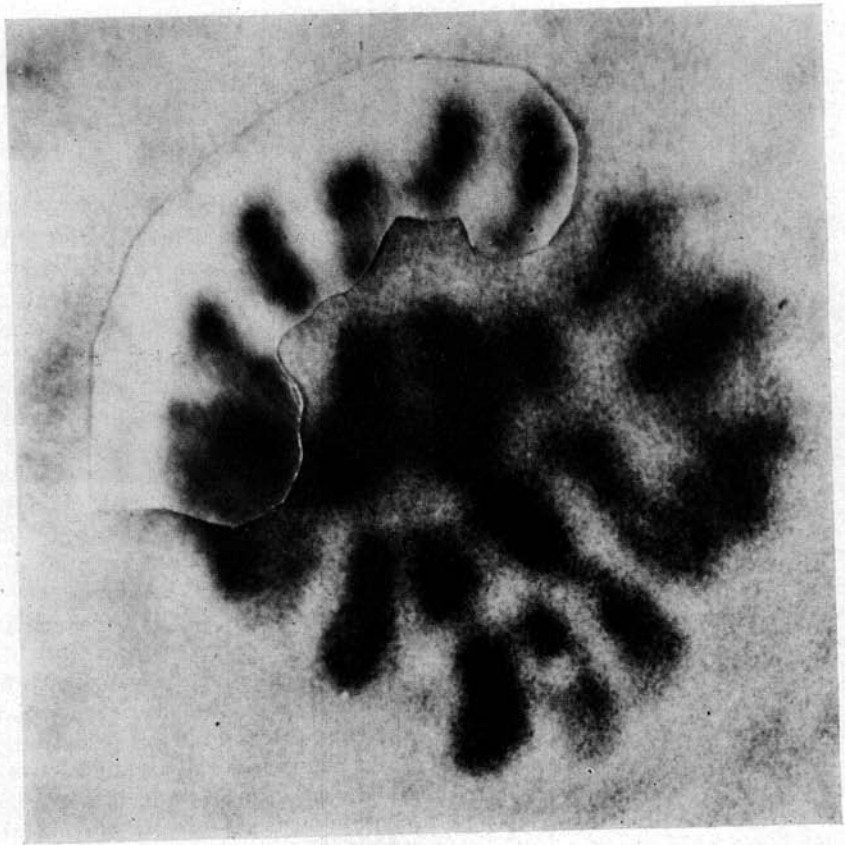

FIG. 1.—Composite photomicrograph of two focal planes of a nuclear division figure of *Rhizoclonium* X8700.

recommended that a minimum time of 20 minutes be given for this stain. The slides may remain in this stain for 24 hours without evident harmful results.

2. *45% Acetic Acid*

This is a destaining solution, and the length of time the slide is in this solution will depend on the length of time the material was in aceto-carmine. If the slide remained in the aceto-carmine for 20 minutes, it is recommended that two minutes in this solution is sufficient.

3. *Glacial Acetic Acid*

This is a destaining agent also, and the length of time the slide is in this acid will depend on the length of time the material was stained. If the slide remained in aceto-carmine 20 minutes and in the 45% acetic acid 2 minutes, one minute in this acid is sufficient. (Note: the amount of staining and destaining will necessarily vary for different types of algae.)

4. ½ *Glacial Acetic Acid* ½ *n Butyl Alcohol*

The slide should remain for 10 minutes in this solution

5. *n Butyl Alcohol*

The slide should remain for 15 minutes in this solution.

6. *Light Green in Clove Oil*

An optional counterstain for making whole mounts of algae.

7. ½ *n Butyl Alcohol* ½ *Xylol*

The slide should remain for a minimum of 10 minutes in this solution. No harmful effects are noted if the slide remains for several hours.

8. *Xylol*

Several changes of xylol are desirable.

The slides may be mounted in balsam for a permanent mount directly from the xylol.

The results from this method have been most gratifying when using plankton forms and many filamentous Chlorophyta. While experimenting with this method, species of *Rhizoclonium* and *Cladophora* were used, as the nuclei are proportionately large. After the method was mastered, the genera of *Spirogyra, Zygnema, Oedogonium, Stigeoclonium* and *Mougeotia* were tried with good results.

In *Cladophora* and *Rhizoclonium* many stages of nuclear division were observed.

In the Myxophyceae excellent results were obtained using *Oscillatoria, Lyngbya, Chroococcus, Merismopedia* and *Gloeotrichia.* Many of the diatoms were processed and excellent results were obtained.

## Bibliography

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