

## NOTES ON MICROSCOPIC TECHNIQUE.

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During the past few years I have been using very successfully a method of staining a number of slides at one time, a description of which may be of interest to others who have occasion to prepare large numbers of slides for class use or for research. The principal features of the method were suggested to me by Miss Louise Sawyer of the Department of Biology of Beloit College.

As shown in the illustration, the slides are held between the coils of a brass spring about an inch in diameter, made of No. 13 wire and wound with the coils in contact. By holding the spring in the left hand and forcing the first two coils apart with the thumb nail, the first slide may be inserted. After this, pressure applied by the thumb upon the slide just inserted separated the coils for the reception of the next slide.

As staining jars, I am now using Bausch and Lomb preservation jars No. 15166 holding 600 c.c., but Stender dishes about 100 mm. deep might prove to be more satisfactory. Vessels to contain stains in which the slides rest for a time (such as safrannin) are more economical of stain if larger.

A coil long enough to hold 12 to 15 slides has been found to be most satisfactory. The spring is kept uppermost until the final xylol is reached, when the spring is reversed, allowing the slides to be pulled out one at a time for mounting. It is easy to hold the rest of the slides with one hand while removing a slide with the other.

The spring I am using was made by Orr and Lockitt, Chicago; a spring about 18 inches long cost 65 cents at that time. Any hardware dealer ought to be able to obtain such a spring.

I have found it desirable to use 3 jars of 95 per cent. alcohol as well as 3 jars of xylol in the series of reagents through which the slides are run. As the alcohol becomes loaded with stain or water, the lowest grade is discarded, each of the others is reduced one grade and the third jar refilled with pure alcohol. The same scheme is used for xylol. By this means, one always has one vessel of pure reagent. Economy of reagents and efficiency of work are facilitated.

Balsam may be kept from spreading beyond the cover-glass and leaving a halo on the finished slide by wiping off the slide with an absorbent cloth close to the sections before putting on the cover-glass. The balsam will then spread to the edge of the cover-glass and stop.

A small amount of valuable material may be made to serve for a larger number of slides, smaller covers may be used, sections may be better oriented and worthless sections discarded if sections are examined just after the paraffin ribbons are stretched. Desirable sections may be cut out by rocking a round-edged scalpel. By laying a new slide smeared with fixative on the table in close contact with the original slide, the sections may be transferred to the new slide with the point of a scalpel, after adding a few drops of water to facilitate the moving of the sections. The sections may be more easily examined while in the paraffin if a little Magdala red is added to one of the higher alcohols in which the material is dehydrated previous to imbedding. The small amount of stain absorbed will not affect future staining operations.

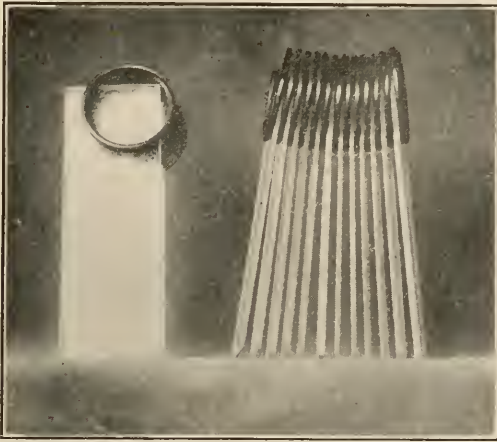
Female gametophytes in pine ovules usually shrink greatly when fixed and imbedded. This may be almost entirely obviated by cutting a slab off each side of the ovule before it is fixed. A Gillette razor blade is very satisfactory, since on account of its thinness it does not crush the material.

Seeds of the pinyon pine (*Pinus edulis*) are very satisfactory to illustrate the gross anatomy of the gymnosperm seed, since they are very large and easily dissected. The gametophyte and contained embryos or the embryos alone may be dissected out, soaked in water a short time, fixed and imbedded. They cut very easily.

A modification of Land's Fixative (See *Botanical Gazette*, Vol. LIX, page 397), has been used very successfully for refractory sections that will not adhere readily with egg albumen. Land's fixative dries very quickly, causing the liquid added to float the sections to spread with difficulty. By using the following formula, the liquid spreads as easily as with egg albumen:

2% gum arabic in water.....	50 c. c.
Glycerin .....	50 c. c.
Sodium salicylate .....	1 gram.

Use as egg albumen. Float sections on water slightly yellow with potassium dichromate. Stretch over warm plate. Melting the paraffin does not impair the efficiency of the fixative. When aqueous stains are used, no previous treatment is necessary; but when alcoholic stains only are to be used, it is best to set the slides for a short time in water to dissolve the excess of fixative adhering to the slide. Otherwise this precipitate will take the stain and spoil the appearance of the slide.



Method of holding microscopic slides in brass springs for staining.

This is best done before the paraffin is removed from the slides. The slides should be re-dried.

A hot-plate for stretching paraffin ribbons that is a great improvement over the old copper plate and gas flame may be made by putting an incandescent lamp in a box and making a glass lid. The heat is uniform. The glass plate gives better contact, though it is better to fill the space between the slide and the glass lid by putting a drop of water on the lid before placing the slide on it. A small box may be made of an ordinary chalk box, the sliding lid of which is replaced by a discarded photographic plate or other piece of glass. It is easier to remove the slides, however, if the lid is flush with the sides of the box.

