

# A Simple Method for Orienting Very Small Specimens for Paraffin Sectioning

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## ABSTRACT

A simple method for the precise orientation of very small specimens for paraffin sectioning is described. The orientation is accomplished under magnification as the paraffin solidifies along a controlled temperature gradient.

## EXPERIMENTAL TECHNIQUES

The following method was developed for sectioning megagametophytes of *Selaginella* and *Isoetes* (roughly 0.5 to 1 mm in diameter) but should prove useful for very small seeds or any seeds in which the external indications of symmetry are difficult to discern in solid paraffin. The essence of the method is simply to cast the infiltrated specimens into a very flat bottomed embedding vessel and arrange the material such that the intended plane of sectioning will be parallel to the bottom of the embedding vessel. Very suitable casting vessels can easily be made of heavy gauge aluminum foil formed upon a block of wood.

In the case of casting multiple specimens in the same vessel, it is essential to establish a temperature gradient in the vessel such that cooling and solidification begins at one end of the vessel and proceeds to the other at a pace convenient to the technician as he or she orients the specimens in the forming crystals of paraffin. This gradient is easily established by warming one end of a suitably massive, smooth, flat piece of metal over a low flame on a Bunsen burner to just above the melting point of the paraffin. The heated metal may be placed upon a piece of cardboard of such dimension that it may be easily moved about on the stage of a dissecting microscope. The casting vessel is placed on the part of the surface which is above the melting point of the paraffin and the specimens spaced appropriately. The vessel is slid toward the cooler part of the unevenly heated surface until the edge of the vessel encounters the freezing point of the paraffin. The specimens are oriented with a hot dissecting needle as the freezing front of paraffin crystals advances across the bottom of the vessel. With very small specimens this can best be done with the aid of a dissecting microscope.

The slab of paraffin produced is subdivided into single specimen blocks in the usual manner. The specimens are mounted on wood blocks and oriented in the microtome such that the surface of the paraffin block formed by the bottom of the casting vessel is presented throughout its travel parallel to and equidistant from the knife edge. As the specimen advances and begins to encounter the knife, fine adjustments of the orientation of the specimen may be made such that in a very few strokes the entire face of the paraffin block will be cut by the knife.

**Equipment Needs**

The equipment includes a dissecting microscope, a Bunsen burner, and a metal block suitable for establishing the temperature gradient through the melting point of the paraffin. I use a rectangular block of steel about 5 cm x 1.9 cm x 15.2 cm for this purpose, but other materials and/or dimensions would undoubtedly serve as well. Better conductors of heat such as brass or aluminum probably would not work so well because the temperature gradient would be less sharp. The block of steel which I use happens to have been machined on all surfaces, but minimally only one surface need be smooth and flat and the opposite surface flat and parallel to the smooth surface.

**RESULTS AND DISCUSSION**

This technique has been used routinely on large numbers of megagametophytes and young sporelings of *Selaginella* and *Isoetes* with consistently satisfactory results. It should certainly be of equal utility with any other small specimens such as seeds.