Microwave Protocols for Plant and Animal Paraffin Microtechnique

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The microwave oven is a valuable tool for light and electron microscopy for microtechnique labs. Tissue processing times, traditionally taking up to two weeks, have been reduced to a few hours as a result of the implementation of microwave technology (Kok *et al*, 1988, Gibberson and Demaree, 2001). In addition, the quality of the tissue preparations has improved dramatically. Microwave ovens have also evolved since their first use in the laboratory. Early experiments were conducted using relatively crude commercial microwave ovens. Now, labs use microwave ovens with temperature probes, strict control over the magnetron (which generates the microwaves), variable power supplies, chamber cooling, and high microwave field uniformity.

We present in this article our standard paraffin microwave protocol for most plant tissues, and a modified protocol for large samples (up to 1cm) or difficult animal tissues. Animal tissues with a high fat content have proven difficult using standard microwave protocols, so we set out to develop a protocol that would work well with these tissues. We do not have access to hospital tissue processors, and are limited in the range and toxicity of histoprocessing chemicals we are permitted to use. With these restrictions in mind, we developed a new protocol. We present a variety of plant tissues, and the liver, kidney, brain, and lung tissue of *Mus musculus*. The protocols described here should work with any laboratory microwave oven with a temperature probe, power level control, and good chamber cooling.

For the work shown here, we used a Microwave Research & Applications, Inc (BP–111–RS). We chose this oven for the higher microwave energy output, high field uniformity, and no water load are requirement. For all steps in the animal protocol, the microwave oven was set to 55% power (750 watts). For all steps in the plant protocol, the microwave oven was set to 45% power (650 watts). All tissues were placed in 10ml of solution in a 20ml glass scintillation vial (Fisher 3-338-E). Sample vials were placed in a 400 ml water bath (Rubbermaid container size 11) for all microwave steps.

Animal tissues were dissected in PBS and placed into ice-cold 10% formalin. All animal samples were processed in the microwave according to the schedule in Table 1. Eosin was added to the 100% ethanol dehydration step to mark the tissues and make them easy to find and orient when in solid paraffin. Isopropanol was used as an intermediate solvent because it is very responsive to microwaves, and it is a less toxic alternative to the traditional intermediates, such as xylene. Tissues were infiltrated with Kendall Paraplast Plus with DMSO (Fisher 12-646-111). Samples were embedded the same day they were processed, then sectioned at 8 μm and stained using a modified Mayer's Hematoxylin (Sigma # MHS16) and eosin Y stain (Ruzin, 1999).

Table 1: Animal Tissue Microwave Processing Schedule

Step	Time (min)	Sample Temp ˚C	Sample Bath ˚C
1. 10% Formalin	30m	37	~ 4-6
2. 50% EtOH	30m	67	(RT) ~19-23
3. 95% EtOH	30m	67	(RT) ~19-23
4. 100% EtOH + eosin	10m	67	(RT) ~19-23
5. 50% EtOH:50% Isopropanol	30m	77	(RT) ~19-23
6. 100% Isop	30m	77	(RT) ~19-23
7. 50% Isop:50% wax	30m	77	~ 60
8. 100% paraffin wax	90m	67	~ 60
9. 100% paraffin wax	90m	67	~ 60
10. embed- samples same day	---	---	---

Plant tissues were dissected and placed into FAA (50% ethanol, 10% formalin, 5% acetic acid) on ice until ready to microwave. Tissues were processed in the microwave oven using the protocol in Table 2. Safranin-O was added to the dehydration step to mark the tissue for later orientation when sectioning. Isopropanol also was used as the intermediate solvent. Samples were infiltrated with paraffin, embedded, and sectioned at 8μm using Johansen's Safranin and Fast Green protocol. (Johansen, 1940).

Results for the animal protocol were excellent. Blocks sectioned easily and remained intact with no tissue loss. Refer to microwave fixed samples in Figure 1. Figure 1A, the *Mus musculus* liver sample was taken from the middle of a 5mm cube. Note that the cells are plump, and there is no cracking or shrinkage. Figure 1B shows a kidney sample from *Mus musculus*. The sample measured 2.5 x 5mm when removed from the animal. The image shown was taken from the middle portion of the sample block to demonstrate the degree of fixation and penetration of the solvents and paraffin. Note the high quality of preservation of the glomerulus. Figure 1C shows the brain tissue of *Mus musculus*. The sample measured 3 x 5mm (brain halved along median sagittal plane). Figure 1D is an image of *Mus musculus* lung tissue. An entire lung, measuring 2 x 4mm, was fixed and processed using the animal microwave protocol. Note the alveoli remained open and intact, and the high quality of preservation of the epithelial cell layer.

All plants tested responded very well using the plant microwave oven protocol. Refer to Figure plate 1 for results. In the *Coleus* stem cross section (Figure 1E), note the epidermal layer remained turgid and continuous, the collenchyma tissue remained intact, and the surrounding tissue was not torn. In Figure 1F, *Capsella bursa* floral structure, the gynoecium remained plump

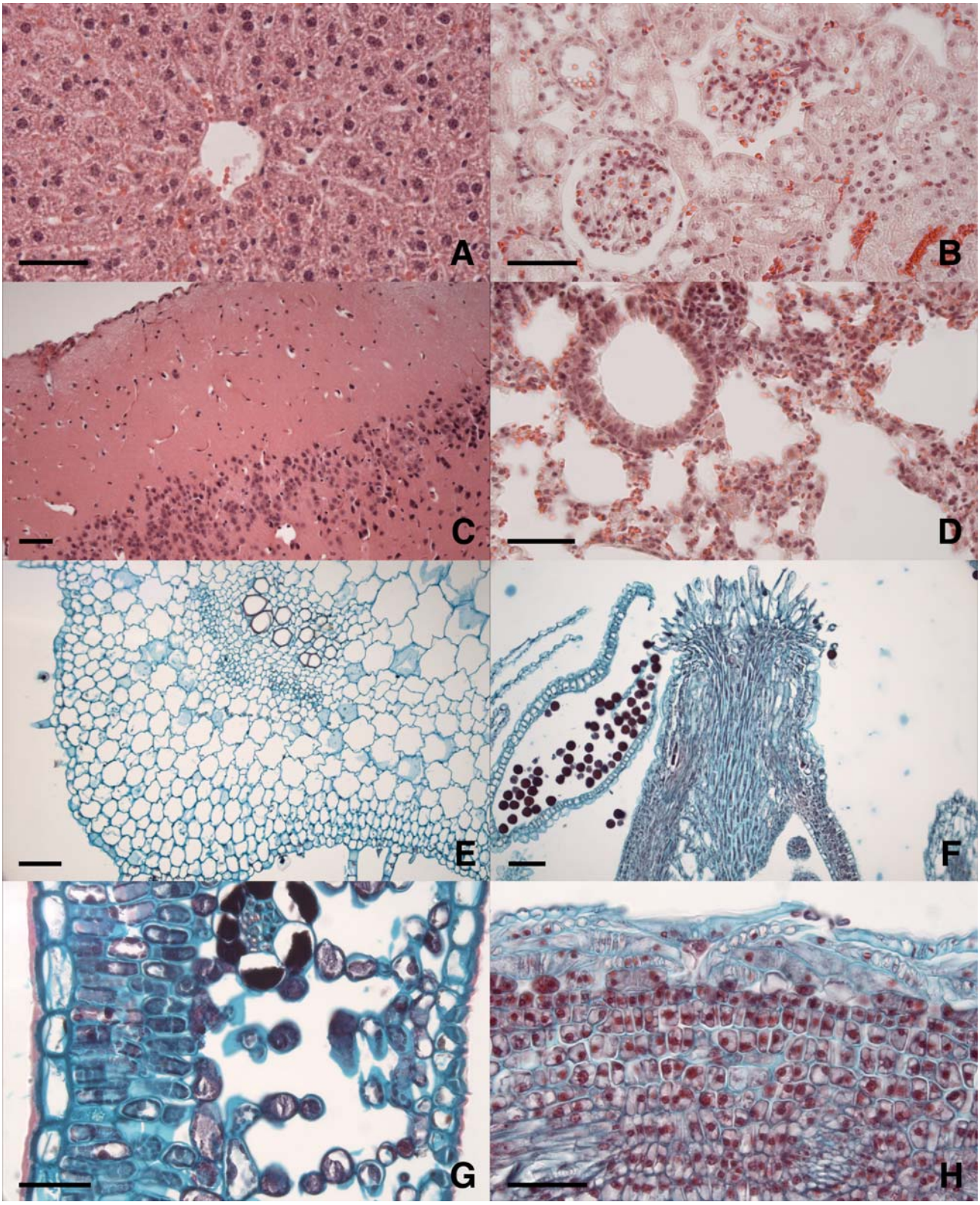

Figure 1. Examples of various animal and plant paraffin-processed tissues. All animal tissue shown was from *Mus Musculus*, sectioned at 6µm, and stained with H&E. All Plant tissue shown was sectioned at 8µm and stained with Johansen's Saf/FG. **A.** Liver. **B.** Kidney cortex. **C.** Brain cortex. **D.** Lung. **E.** Coleus stem xs. **F.** *Capsella bursa* floral ls. **G.** California Bay Laurel leaf xs. **H.** *Olea europaea* leaf xs. For all images, scale bars = 50µm.

and intact. This tissue is particularly delicate and often is subject to distortion during the dehydration process. Even the stigmatal surface was well preserved. In the California bay leaf cross section of (Figure 1G), the epidermal wax layer was preserved as a continuous layer. The guard cell pair and associated mesophyll air space were well preserved, and the palisade parenchyma cells remained tightly packed with no cracking. As a demonstration of the efficacy of microwave processing on dense, recalcitrant tissue, the olive (*Olea europaea*) leaf sectioned beautifully, including the epidermal scales.

Table 2: Plant Tissue Microwave Processing Schedule

Step	Time (min)	Sample Temp ˚C	Sample Bath ˚C
1. FAA	15m	37	~ 4-6
2. FAA	15m	37	~ 4-6
3. FAA	15m	37	~ 4-6
4. 70% EtOH	5m	67	(RT) ~19-23
5. 85% EtOH	5m	67	(RT) ~19-23
6. 95% EtOH	5m	67	(RT) ~19-23
7. 100% EtOH + safranin O	5m	67	(RT) ~19-23
8. 100% EtOH + safranin O	5m	67	(RT) ~19-23
9. 50% EtOH:50% Isopropanol	5m	77	(RT) ~19-23
10. 100% Isop	5m	77	(RT) ~19-23
11. 50% Isop:50% wax	10m	77	~ 60
12. 100% paraffin wax	30m	67	~ 60
13. 100% paraffin wax	30m	67	~ 60
14. 100% paraffin wax	30m	67	~ 60
15. 100% paraffin wax	30m	67	~ 60
16. 100% paraffin wax	30m	67	~ 60
17. embed- samples same day	---	---	---

The two protocols described here differ in three key elements. First, the plant protocol has shorter times than the protocol for animal tissues. Animal tissue treated at short step times formed an outer crust of paraffin-infiltrated tissue, while the center of the sample remained unfixed and thus not well infiltrated. Secondly, the animal protocol has fewer steps from fixation to final embedding. While animal tissue responded well to this abbreviated protocol, plant tissues were poorly infiltrated. Finally, animal tissue responded much better using a higher power setting on the microwave oven. Depending on the type of plant, some tissue types did better on an even lower power setting, such as *Arabidopsis thaliana* (Schichnes *et al*, 2001).

Why do plant and animal tissues have such differing paraffin processing requirements? Perhaps it relates to the cell wall present in plant tissues but not in animal tissues. Even when the plant tissue is dead, this "apoplastic transport pathway" may function to draw solutions into the plant tissue. Made up of cell walls and intracellular spaces, the apoplastic portion of the plant acts like many wicks, allowing solvents to penetrate deep into the tissue via liquid surface tension and cohesion. Plant tissues, therefore, might tolerate shorter step times because the solvents penetrate the tissues more quickly and thoroughly. In animal tissues, however, solvents would not penetrate as deeply with shorter step times. This, in turn, would lead to a thin layer of processed tissue, surrounding a core of raw, unprocessed tissue; which we have observed.

Dramatic changes in alcohol concentration usually is associated with cell damage (due to surface tension phenomena), and poor paraffin infiltration. However, our animal microwave protocol has many fewer steps than the plant tissue protocol. One possible explanation to the success of this rapid protocol is that since animal tissue has no apoplastic pathway, the slow penetration rate of solvents throughout the tissue could act as a natural gradation of solvent steps. With a much more continuous gradient of solvent concentration, animal tissue shows little stress-induced damage. Plant tissues react to dramatic steps by shrinking and cracking, resulting in poor paraffin infiltration and difficult sectioning, which we observed.

In dense plant tissue, such as the small, tightly packed cells of *Zea mays* developing female inflorescence, a higher power level yielded better results. Conversely, in plant tissues that are loosely packed, such as leaf spongy mesophyll, or have little supportive structure, such as most *Arabidopsis thaliana* tissues, lower power settings (450 watts) yielded better results (Schichnes et al, 2001). Perhaps animal tissue act similarly, and thus respond better with a higher power setting, as we observed when designing these protocols.

Plant and animal tissues vary on many levels, in terms of structure, function, and content. We suggest that there cannot be a "one size fits all" microwave protocol that can span the two kingdoms. These differences should be considered when designing paraffin-processing protocols. The two protocols described here are a good place to start your investigation.

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