

## **Laboratory for Advanced Electron and Light Optical Methods**

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### **Procedure for De-paraffinizing Tissues**

**1. Applications and Objectives:** On some occasions, it is necessary to retrieve information from samples previously fixed and embedded for standard histological examination. This situation is not uncommon in a clinical setting where bioptic samples are collected (e.g., tumors) and all the tissue is embedded in paraffin. After light microscopic examination, the clinical staff determines that ultrastructural examination is necessary for absolute identification of the pathology present. The electron microscopy laboratory staff is then confronted with attempting to supply information from a sample that may have been suboptimally aldehyde-fixed (4% formaldehyde), dehydrated, and then heated for a prolonged period during paraffin infiltration.

The technique described below is modified from Weidmann et al. (1987).

### **2. Materials Needed:**

xylene

ethanol dehydration series

2% osmium

0.2 M Sorenson's sodium phosphate buffer, pH 7.2-7.4

Spurr resin (6.3 recipe as described in the resin section)

### **3. Procedure:**

- 3.1. Cut piece of tissue out of the paraffin block, removing as much excess paraffin as possible.
- 3.2. Place in 100% xylene for 1-2 hr, changing xylene several times until no evidence of paraffin remains.
- 3.3. Rehydrate tissue:
  - 100% ethanol: two changes over 60 min
  - 95% ethanol: two changes over 30 min
  - 75% ethanol for 15 minutes (or overnight at 4° C)
  - 50% ethanol for 15 minutes
  - distilled water: two changes over 10 min
- 3.4. 1% osmium in 0.1 M phosphate buffer for 1 hr.
- 3.5. Rinse in distilled water two times (5 min each). Dehydrate and embed in Spurr resin as described for routine TEM sample preparation.

**4. Results Expected:** Such material, usually being suboptimally fixed in 4% formalin and then washed for extended periods of time before being embedded in paraffin, will usually not look very good. Much of the cytoplasmic detail will have been leached out and membranes will often be severely disrupted. The heating steps involved in paraffin embedding will also have coagulated much of the cytoplasm. This procedure is a viable option if no more tissue can be obtained from important cases because proteinaceous components such as cytoskeletal filaments, specific cellular granules, and junctional complexes between cells can still be identified in most cases. This type of sample will not often yield publishable results, but can still provide useable diagnostic information.

**5. Cautionary Statements:** Handle and dispose of xylene according to MSDS

information. Xylene is a toxic and potentially carcinogenic material and must be handled appropriately. Other cautions concerning routine TEM processing apply.

**References:**

Weidmann, J., M. Freund, and B. McGeever-Rubin. 1987. Comparative light and electron microscopy of paraffin-embedded tissue: An assessment of three methods. *J. Histotechnol.* 10:163-166.

**Supplementary reference:**

Chien, K., R.L. Van de Velde, and R.C. Heusser. 1982. A one-step method for re-embedding paraffin embedded specimens for electron microscopy. p. 356-357, *Proc. Electron Microsc. Soc. Amer. 40th Annual Meeting*, San Francisco Press, Inc., San Francisco, CA.