# Chapter 2

# Microwave-Assisted Processing and Embedding for Transmission Electron Microscopy

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## Abstract

Microwave processors can provide a means of rapid processing and resin embedding for biological specimens that are to be sectioned and examined by transmission electron microscopy. This chapter describes a microwave-assisted protocol for processing, dehydrating and embedding biological material, taking them from living specimens to blocks embedded in sectionable resin in 4 h or less.


**Key words** Transmission electron microscopy, Resin embedding, Thin sectioning, Immunocytochemistry, Rapid processing, Microwave

## 1 Introduction

### 1.1 History

Rapid processing of tissues for histology offers important advantages for rapid diagnosis, convenience and efficiency. The earliest methods for decreasing processing times involved heating specimens [1, 2]. Introduction of the microwave processor into the laboratory offered a more controlled approach to heating tissues and cells. By penetrating the biological material, microwaves overcame the limitation of poor heat conduction and thus produced more even heating throughout the processed specimens. Introduced in 1970 for histological processing [3] microwave fixation produced satisfactory results with few artifacts in mouse and fresh human postmortem tissues [3]. The idea of heating tissues in a constant volume bath of liquid to stabilize the temperatures inside the microwave chamber was introduced in 1978 [4] when Login performed a study to compare the effects of heating tissues in different solutions. Heating tissues in saline or Zenker's solution (a fixative used by histologists that contains mercuric chloride and acetic acid) produced better results than if tissues were heated in formalin [4]. Enzyme reactivity and immunoreactivity was preserved in microwave-fixed tissues [5], microwave-assisted heating of sections

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was discovered to be an efficient method for antigen retrieval [6], and immunolabeling times could be shortened using microwaves [7–9]. Microwave irradiation was also found to decrease decalcification rates of human temporal bones with no detectable adverse effects on ultrastructure or antigenicity [10–13]. The microwave processor thus became a routine tool in the histology laboratory for heating biological specimens and speeding up processing times [14–18].

Introduction of microwave technologies to the electron microscopy laboratory were initially restricted to methods involving specimen heating [5, 19]. Although the microwave-induced heating produced useable preserved specimens, the ultrastructural damage was not suitable for critical work [5, 19, 20]. Microwave-induced heating in the presence of aldehyde gradually took over as a viable approach to rapid fixation for electron microscopy [20–23]. Microwave processing under more careful temperature control was achieved using water loads in the chamber of the processor, or by shielding the tissues with cooling jackets containing circulating water [24, 25]. Careful evaluation of microwave-assisted processing of biological material began to reveal improved morphology when compared with conventional processing methods performed at ambient temperature [26–30]. Examination of microwave-processed bone revealed improved ultrastructure and antigen retention [12, 31].

The technical developments that allowed biological material to be exposed to microwaves without being heated [29], opened a controversy that is still not completely settled, that of whether there is a "microwave-only effect" independent of temperature. Early studies suggested that specimen heating caused by exposure to microwaves was responsible for the decrease in preparation times and improved ultrastructure [20, 32]. However, more recent studies using more carefully controlled conditions seem to indicate a role for microwaves in the absence of heat in the improvements of processing times and ultrastructure [29, 33]. Electromagnetic effects of microwaves on cellular membranes, supporting a microwave-only effect, have been demonstrated in *Escherichia coli* bacteria [34].

### 1.2 Contemporary Uses of Microwave Processors in Biomedical Research

Microwave processors have become essential tools in the histology laboratory and have been incorporated into routine protocols. Microwaves are used to assist in specimen fixation [16, 35, 36], paraffin embedding [37, 38], antigen retrieval [6, 39, 40], staining [15, 41, 42], immunolabeling [7, 9, 39, 43–47], and in situ hybridization [48, 49]. Microwave processors are also being used for rapid bone decalcification [10–13, 33] (*see* Fig. 1).

Similar reports of microwave-assisted processing for electron microscopy indicate rapid processing times, improved specimen morphology and increased antigenicity of specimens being used for immunocytochemical experiments [21, 26, 28, 29, 31, 47, 50–54]. Microwave assisted resin polymerization has been used to embed

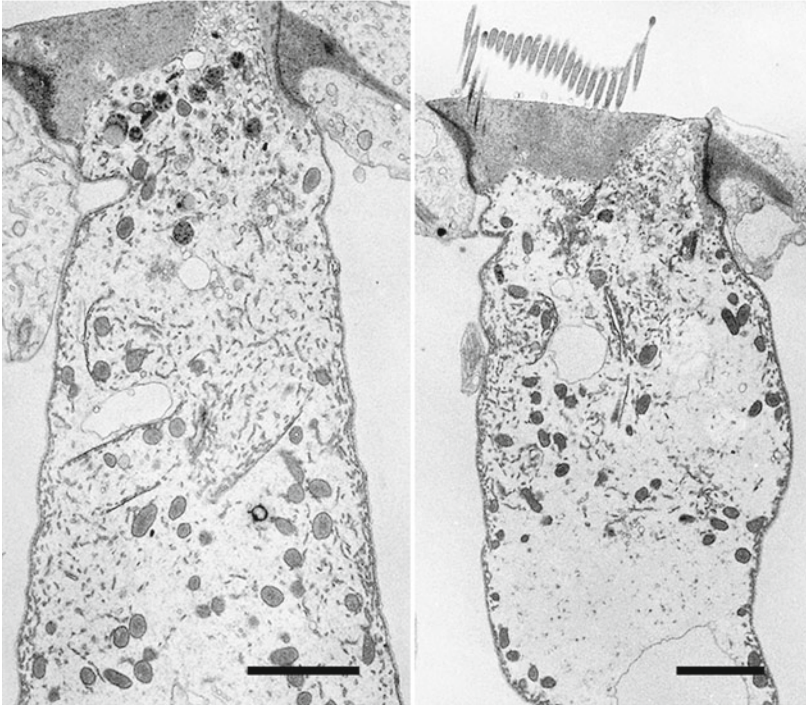

**Fig. 1** Outer hair cells from the mouse inner ear. The whole inner ear, or cochlea was removed after perfusion fixation and then decalcified in 125 mM EDTA in a microwave processor. The whole cochlea was embedded in epoxy resin following the microwave-assisted protocol detailed in this chapter. The tissues were embedded in resin using heat polymerization and thin sections were examined in a TEM. Scale bars = 2 μm

thawed, thin cryosections [55], and diluted antibodies have been microwave irradiated to increase their labeling efficiency for EM immunocytochemistry [46]. As a result of these reports, and with the inclusion of microwave processing in the curricula of practical courses, electron microscopy laboratories are adapting the microwave processor for use in routine protocols.

### *1.3 A Routine Microwave-Assisted Processing Protocol for Electron Microscopy*

The protocols described in this chapter have been extensively tested in different laboratories using different microwave processors and have been shown to yield reproducible results. The only variable we have discovered is with the Epon substitute available from different suppliers. The Spurr-Epon recipe we have provided will polymerize differently if Eponate 12 is not used. However, it appears that all Epon substitutes can be used, but the final result may require some adjustment to optimize the hardness of the resin. We recommend using complete kits from a single supplier, and not mixing ingredients from different suppliers. We also recommend testing all resin mixtures before using them to embed specimens.

The complete protocol can be applied as is, or, as is the case in my laboratory, parts of the protocol can be incorporated into existing long protocols. For example, we occasionally perform dehydration

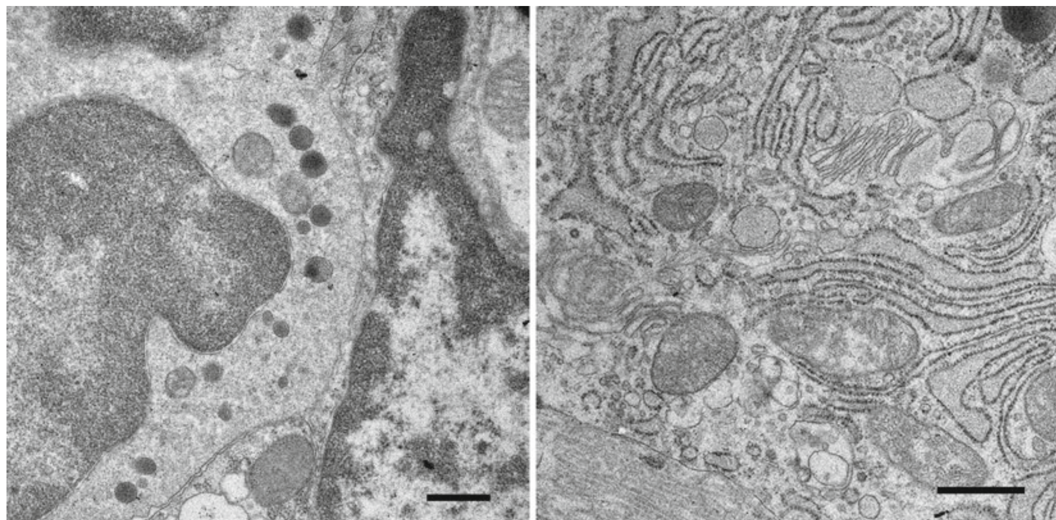

**Fig. 2** Cells in the rat round window membrane, a membrane, three-cell layers thick, that separates the middle ear cavity from the inner ear, or cochlea, in mammals. The ability of drugs to pass through this membrane is important for delivery of therapeutics to the inner ear for treating hearing disorders. The membrane was removed from a freshly dissected cochlea and immersion fixed in 2.5 % glutaraldehyde buffered with 100 mM sodium cacodylate (pH 7.2). The tissue in fixative was irradiated in the microwave processor following the protocol described in this chapter. The membrane was cut into pieces and further processed following this protocol and finally embedded in resin, which was polymerized overnight at 60 °C. Thin sections were examined in a TEM. Scale bars = 500 nm

and resin infiltration using the microwave processor but perform the final resin polymerization at 60 °C in a regular oven. We also use rapid resin polymerization to re-embed polymerized specimens that need reorienting for sectioning. Examples of microwave assisted processing can be examined in Figs. 2, 3, and 4 in this chapter. All were processed into epoxy resin using microwave-assisted fixation, dehydration, and resin infiltration using the protocol outlined below and annotated in Table 1(a). All specimens were infiltrated using the epoxy resin formulation documented in Table 2(c). The specimens illustrated in Fig. 2 were polymerized in resin using the microwave protocol described in Subheading 3.6. Specimens illustrated in Figs. 3 and 4 were polymerized in resin using an overnight exposure to 60 °C.

## 2 Materials

### *2.1 Equipment*

1. A microwave processor designed for laboratory use with the following features [37, 38]:
   (a) Magnetron prewarming.
   (b) Variable wattage.

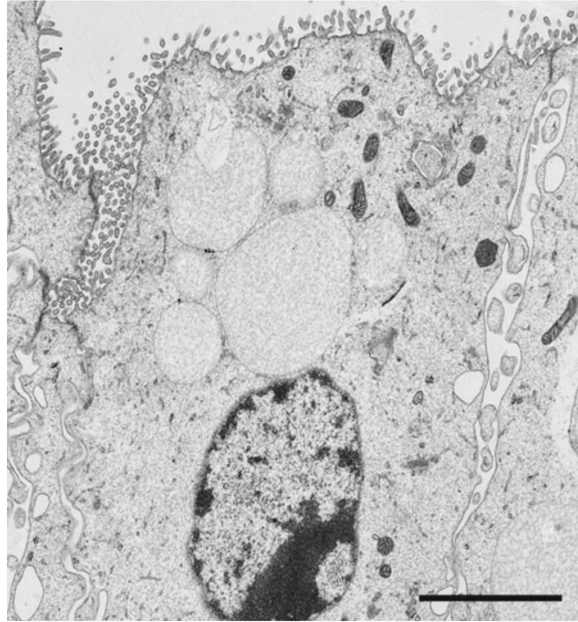

**Fig. 3** A TEM image of a Hensen's cell dissected from a mouse cochlea. A row of Hensen's cells runs alongside the organ of Corti in the mammalian inner ear and assist in protecting the sensory cells of the auditory organ by secreting annexin A1in response to corticosteroid treatment [30]. A strip of cells was dissected from a fresh mouse cochlea and immersion fixed in 2.5 % glutaraldehyde buffered with 100 mM sodium cacodylate (pH 7.2). The cells were further processed using the microwave-assisted protocol described in this Chapter. Scale bar = 2 μm

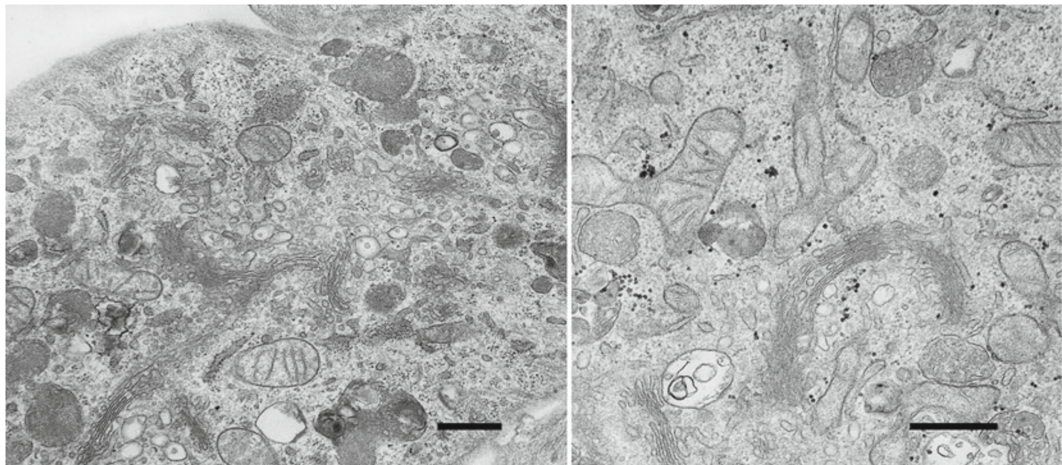

**Fig. 4** Two images of cells used in a study examining the uptake and intracellular fate of nanoparticles with therapeutic applications [63, 64]. The cells were grown in vitro, incubated with nanoparticles and fixed by immersion in 2.5 % glutaraldehyde buffered with 100 mM sodium cacodylate (pH 7.2). The cells were then scraped, centrifuged into a pellet and processed using the protocol described in this chapter. The images show regions of the cell containing the Golgi complex. Mitochondria and membrane-bound organelles can also be observed. Scale bars = 500 nm

**Table 1**
**Annotated microwave embedding protocols**

| Time | Solution | Microwave | Temp limit |
|---|---|---|---|
| Unless noted otherwise, all microwave irradiations are carried out with the processor operating at full power and containing a full water load | | | |
| (a) Embedding in Epoxy Resin (for specimens already chemically fixed). Use resin formulations A or B from Table 2 | | | |
| Time | Solution | Microwave | Temp limit |
| 10 s | Fixative | Yes | 30 °C |
| 20 s | Fixative | No | n/a |
| 10 s | Fixative | Yes | 30 °C |
| 5 min | Buffer | No | n/a |
| 40 s | 1 % osmium tetroxide | Yes | 37 °C |
| 30 s | Water | No | n/a |
| 40 s | 50 % Acetone | Yes | 37 °C |
| 40 s | 70 % Acetone | Yes | 37 °C |
| 40 s | 80 % Acetone | Yes | 37 °C |
| 40 s | 90 % Acetone | Yes | 37 °C |
| 40 s | 100 % Acetone | Yes | 37 °C |
| 40 s | 100 % Acetone | Yes | 37 °C |
| 15 min | 1:1 Acetone–resin | Yes | 45 °C |
| 15 min | 100 % resin | Yes | 45 °C |
| 15 min | 100 % resin | Yes | 45 °C |
| 10 min | Resin | Yes | 101 °C |
| 80 min | Resin | Yes (50 % Power) | 101 °C |
| (b) Embedding in LR White Resin (for specimens already chemically fixed). Use LR White Resin (formulation C from Table 2) | | | |
| 10 s | Fixative | Yes | 30 °C |
| 20 s | Fixative | No | n/a |
| 10 s | Fixative | Yes | 30 °C |
| 5 min | Buffer | No | n/a |
| 30 s | Water | No | n/a |
| 40 s | 50 % Ethanol | Yes | 37 °C |
| 40 s | 70 % Ethanol | Yes | 37 °C |
| 40 s | 90 % Ethanol | Yes | 37 °C |
| 40 s | 100 % Ethanol | Yes | 37 °C |
| 40 s | 100 % Ethanol | Yes | 37 °C |
| 15 min | 1:1 ethanol–resin | Yes | 45 °C |
| 15 min | 100 % resin | Yes | 45 °C |
| 15 min | 100 % resin | Yes | 45 °C |
| 45 min | Resin | Yes | 95 °C |

   (c) Forced gas extraction system.
   (d) Thermocouple temperature probe.
   (e) Load cooler.
   (f) Programmable presets.

2. An ultramicrotome for thin sectioning resin-embedded biological specimens.
3. A transmission electron microscope (TEM) operating at 80 kV for examining thin sections.
4. A 60 °C oven is recommended for heat polymerization of resin.

**Table 2**
**Resin formulations for microwave-assisted polymerization**

| | |
|---|---|
| It is possible to prepare the following resin formulations without adding the catalyst. The resin mixtures can be stored in small aliquots at –20 °C for extended periods of time without the risk of the resin polymerizing (*see* **Note 23**). When the resin is ready to use warm an aliquot to room temperature and add catalyst dropwise from glass Pasteur pipettes. The numbers of drops to be added has been calibrated using glass Pasteur pipettes (1 drop = 0.01 g) | |
| (a) SPURR-EPON Resin (from [51]) | |
| To make approx 20 ml mix the following together in a tube: | |
| ERL 4206 | 2.5 g |
| DER 736 | 1.0 g |
| NSA | 6.5 g |
| Eponate 12 | 6.25 g |
| DDSA | 3.25 g |
| NMA | 3.0 g |
| Store aliquots frozen in glass or polypropylene tubes | |
| Before use, thaw and add: | |
| DMAE | 6 drops |
| DMP-30 | 8 drops |
| (b) EPON Resin (from [50]) | |
| Prepare and mix: | |
| *Solution A:* Epon 812 (1.86 g) + DDSA (2.4 g) | |
| *Solution B:* Epon 812 (2.48 g) + NMA (2.17 g) | |
| Mix equal amounts of solutions A and B and add BDMA, 4 drops per ml | |
| (c) Epon Resin (a recipe for making large batches) | |
| Mix the following in a large glass jar: | |
| NMA | 110 ml |
| DDSA | 130 ml |
| Eponate 12 | 230 ml |
| Mix the ingredients well and pour the resin into small tubes in aliquots of 4–6 ml, and store frozen | |
| To use, warm an aliquot of resin and add 4 drops of BDMA per ml. Use immediately and discard unused resin | |
| Preparing and storing resin this way will ensure that resin ingredients are not wasted and all the tubes will have resin of the same consistency. The mixture can be tested before use by polymerizing one tube of resin. All subsequent tubes of resin will have the same qualities as this first tube, so long as the same amount of catalyst is added | |
| (d) LR White | |
| This resin is supplied premixed. Warm to room temperature before use | |

### 2.2 Consumables

1. Glass or plastic specimen vials.
2. Eppendorf tubes (1 ml and 0.5 ml size).
3. Two 500 ml glass beakers.
4. Rubbermaid sandwich boxes.

**Table 3**
**Resin components used in microwave-assisted polymerization**

| (a) Spurr's resin | (b) Epon 812 | (c) LR White |
|---|---|---|
| Comprising:<br>ERL 4206<br>DER 736<br>NSA<br>DMAE | Comprising:<br>Epon 812 substitute<br>DDSA<br>NMA<br>BDMA | This resin is sold as a premix and should be stored at −20 °C following suppliers instructions |

5. Plastic Pasteur pipettes.
6. Ice buckets.
7. BEEM capsules.
8. Teflon capsule holders or Eppendorf tube racks.
9. ParafilmM® (Parafilm)

### 2.3 Chemicals

1. 2.5 % glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.2).
2. 4 % formaldehyde in 100 mM HEPES buffer (pH 7.0).
3. 2 % aqueous osmium tetroxide.
4. 100 mM sodium cacodylate, pH 7.2.
5. 100 mM HEPES buffer, pH 7.0.
6. Increasing concentrations of acetone in water (50 %, 70 %, and 90 %).
7. Dry acetone.
8. Embedding resins for electron microscopy. The resins are available in kit form, and the individual components can be purchased from any electron microscopy supply company (*see* Tables 3 and 2).

## 3 Methods

### 3.1 Setting Up the Microwave Processor

Commercial laboratory microwave processors are recommended for the protocols in this chapter (*see* **Note 1**). The commercially available instruments meet the necessary safety standards required when handling the hazardous materials encountered in biomedical laboratories. Follow the manufacturer's recommendations when installing and operating the microwave processor (*see* **Notes 2** and **3**) and pay special attention when connecting the gas extraction system. It must be attached to an outlet suitable for handling volatile, toxic gasses.

Once the processor has been connected, it is necessary to create a cold spot within the chamber where specimens can be irradiated (*see* **Notes 4–6**). Placing a tank in the microwave chamber through

which water circulates creates the cold spot. The water load will absorb the heat produced by the microwaves and create an area on the chamber floor where microwave irradiation is more evenly distributed. Test the cold spot with either a neon bulb array or an array of small tubes filled with water (*see* **Notes 7–9**).

### 3.2 Chemically Fixing Biological Specimens

Cells and tissues to be processed for examination by electron microscopy are usually cross-linked using chemical agents such as glutaraldehyde or formaldehyde. The biological material is either immersed in the chemical fixative or the fixative is perfused throughout the specimen. Successful cross-linking depends on the chemical fixative to be able to rapidly penetrate and react with cellular components. The exact mechanism by which chemical fixatives operate is still not fully understood (for a review of chemical fixation *see* Chapter 2 of [56]). The mechanisms by which microwaves assist in chemical fixation are even less well understood (*see* **Note 10**). However, many protocols are available that are reported to work successfully. For this protocol, a fixation schedule proposed by Giberson and Demaree [51] is suggested.

#### 3.2.1 Microwave-Assisted Chemical Fixation

1. If the microwave processor has a temperature probe connected to the magnetron, set the temperature limit to 30 °C.
2. Carefully dissect out the target tissue and immerse it immediately in 2.5 % buffered glutaraldehyde. Carefully cut the specimen into 2–3 $mm^3$ blocks using two thin, flat razor blades and transfer the blocks to glass vials containing fresh 2.5 % buffered glutaraldehyde.
3. Cool the vials on ice and transfer them to the cold spot in the microwave chamber.
4. Irradiate for 40 s at 100 % power and transfer them to ice outside of the microwave chamber. Incubate for 5 min.
5. Place the vials back in the chamber and irradiate again for 40 s at 100 % power.

### 3.3 Post-fixation Using Osmium Tetroxide

1. Keep the temperature limit on the processor at 30 °C.
2. Remove the fixative from above the specimen blocks but do not let them dry. Add sodium cacodylate buffer and incubate for 5 min on ice outside the microwave chamber.
3. Remove the buffer and add cold 1 % aqueous osmium tetroxide.
4. Microwave at 100 % power for 40 s.
5. Transfer the vials back to ice outside the processor and incubate for 5 min on ice.
6. Keeping the specimens immersed in the 1 % osmium tetroxide, return the vials to the processor chamber and microwave at 100 % power for 40 s (*see* **Note 11**).

### 3.4 Dehydration

1. Remove the osmium tetroxide and replace with distilled water. This is a quick change only—do not let the specimen incubate for long (*see* **Note 12**).
2. Specimen dehydration consists of exposing the specimens to increasing concentrations of acetone. The dehydration steps are single changes in 50 %, 70 %, and 90 % acetone followed by two changes in 100 % acetone (*see* **Note 13**).
3. For each change in the acetone solution, microwave the specimens at 100 % power for 40 s (*see* **Note 14**).
4. Remove the specimens from the microwave processor after each step and replace the acetone with the next, increasing concentration of acetone. A protocol using LR White will have slight alterations that include substituting ethanol as the dehydration medium (*see* **Note 15**).

### 3.5 Resin Infiltration

1. Set the temperature limit on the processor to 45 °C
2. Replace the 100 % acetone in the specimen vials with a mixture containing equal volumes of 100 % acetone and epoxy resin (*see* box 2 for formulations).
3. Irradiate the vials for 15 min at 100 % power.
4. Remove the acetone–resin mixture and replace with 100 % resin.
5. Irradiate for 15 min at 100 % power.
6. Remove the resin and add fresh 100 % resin.
7. Irradiate for 15 min at 100 % power (*see* **Note 16**).

### 3.6 Resin Polymerization

1. Remove the specimens and place each block individually into a BEEM capsule. The BEEM capsules should have the lids cut off and be firmly held in a Teflon rack. Unique identifier labels can also be placed into the tubes at this stage (*see* **Note 17**).
2. Fill the capsules with fresh resin containing catalyst and cover the top with a small square of Parafilm.
3. Cover the Parafilm with the previously removed BEEM capsule lid and press the lid firmly in place. The aim of this step is to seal the BEEM capsule and keep water from entering the capsule (*see* **Note 18**).
4. Place the Teflon tray containing the BEEM capsules into a Rubbermaid tray and cover the capsules with tap water.
5. Place the tray in the microwave processor, place the temperature probe into the water and set the temperature limit to 101 °C (*see* **Note 19**).
6. Microwave at 100 % power for 90 min (60 min plus 30 min) (*see* **Note 20**). Shorter exposure to microwaves is required for LR White polymerization (*see* **Note 21**).

7. Remove the tray containing the BEEM capsules and hot water (*see* **Note 22**).
8. Remove the BEEM capsules from the water and let it cool.
9. Using a razor blade or BEEM capsule press, remove the plastic mold from the block and prepare for sectioning.

## 4 Notes

1. The methods described in this chapter for rapid fixation, dehydration, and resin embedding have been tested using a regular high-power household microwave oven and a commercially available laboratory microwave processor. For safety reasons, we do not recommend the use of a regular high-power microwave oven. Glutaraldehyde, formaldehyde, osmium tetroxide, and the resins used for embedding biological materials give off toxic vapors that are harmful to humans. They should only be used in well-ventilated areas where the fumes can be immediately dissipated. Acetone is flammable and may be exposed to ignition sources if used in unmodified and unshielded microwave processors.
2. Microwave processors should not be operated if the chamber is empty. Doing so may cause damage to the magnetron.
3. All microwave processors have the ability to heat objects exposed to microwaves. The heating is unpredictable in that contents of containers can become much hotter than the container. Removing objects from a microwave processor should be performed with extreme caution after microwave irradiation and all objects should be treated as burn hazards.
4. Login and Dvorak have extensively covered the calibration of laboratory microwave processors [57].
5. Hot spots are areas within the microwave chamber that result from resonating microwaves generated within the closed chamber. The microwaves produce an uneven distribution of standing waves that accumulate in some regions but not others.
6. Hot spots in the microwave chamber can be detected using a neon bulb array or liquid crystal temperature strips (both available from many EM supply companies). These indicators provide instant, visible maps of hot spots. However, the hot spots can also be identified using an array of small tubes containing equal volumes of water. Place the array on the chamber floor and turn on the processor at full power for a short time (e.g., 5 s). Liquid crystals and neon bulb arrays will immediately light up to indicate hot spots. If tubes of water are used, the hot spots are revealed by increased water temperature in the tubes. Although, not recommended, Styrofoam sheets have been

used to detect hot spots [8]. After irradiation, patches of melted plastic identify the hot spots!

7. The location of the cold spot will remain constant if the water loads remain constant.
8. Although using beakers of water to create a cold spot is obsolete if a commercial laboratory microwave processors is available, it is possible that some users will not have access to an enclosed circulating water load. If beakers of water are being used, Giberson and Demaree [51] recommend temperature changes of more than 10 °C but less than 15 °C in 600 μL volumes of water exposed to microwaves at 100 % power for 40 s. Larger volumes of water can be used for the water load if required, either by increasing the size of the beakers used, or by adding extra beakers of water.
9. The water load can be recycled through a water chiller to help minimize heating within the chamber. A flat chamber placed on the floor of the microwave through which cold water is circulated, can be used to create a cold spot (for example, the Pelco ColdSpot® available from Ted Pella Inc.). Specimen vials are placed on top of this chamber when being irradiated.
10. Microwave-assisted chemical cross-linking using buffered glutaraldehyde or formaldehyde is still not well understood. Earlier studies of this process are difficult to assess because short exposure times to microwaves were used but the biological material was then left soaking in dilute fixative for long periods.
11. Increased contrast can be obtained by replacing the 1 % osmium tetroxide with reduced osmium. An aqueous solution of 1 % osmium tetroxide containing 0.3 % potassium ferrocyanide is added to the specimen blocks and irradiated at 100 % power for 40 s. Cool the specimen vials as described.
12. An *en bloc* staining step using can be inserted after the osmium tetroxide post-fixation. After the osmium solution is removed the specimens are washed in distilled water and then irradiated at 100 % power for 40 s in aqueous 1 % uranyl acetate. Using 1 % uranyl acetate dissolved in 50 mM sodium maleate (pH 5.2) instead of distilled water may help increase specimen contrast.
13. Although the protocol presented here uses a routine dehydration protocol with relatively large increases in dehydrating solution, we have recently observed that embryos processed for embedding in epoxy resin will shrink significantly when transferred directly from 70 % to 90 % acetone (or ethanol). When shrinkage is an issue and must be prevented, we dehydrate specimens using more gradual increases in solvent (e.g., 70 % to 75 % to 80 % to 85 % to 90 %).

14. Large specimen blocks or impermeable materials such as nerve biopsies, yeast, embryos or plant material may require longer soaking times in the dehydration solutions to ensure complete removal of water from the specimens. Such impermeable materials may also require extended exposure to osmium tetroxide and resins during infiltration to enable complete penetration of these substances into the specimen.
15. The suppliers of LR White do not recommend using acetone when dehydrating biological specimens because residual amounts of acetone can interfere with the polymerization process. When using LR White in the microwave assisted protocol, ethanol can be substituted for the acetone without problem.
16. Some tissues and cells are particularly difficult to process, even when not using microwave-assisted methods (*see* **Note 14**). We have encountered difficulties with human nerve biopsies that required much longer exposure to osmium tetroxide than most other tissues. Longer exposure can take place during exposure to microwaves or outside the microwave chamber. We have also encountered difficulties in embedding yeast cells and rodent embryos. In both instances, the problem originated from incomplete infiltration with resin. The most extreme case was with the yeast cells that were first exposed to the preparation protocol described in this chapter. To obtain complete resin infiltration, the cells were taken from the microwave chamber, placed on a rotating table and soaked for an additional 3 days in un-catalyzed resin, changing the resin each day. Embedding was performed in fresh resin containing catalyst [58].
17. Early reports on microwave assisted processing strongly advise against embedding specimens in soft, cylindrical containers such as BEEM capsules. The heat produced in the microwave chamber could result in the capsules melting and deforming. However, if the resin is polymerized with water loads present in the microwave, and if the BEEM capsules are immersed in water (it is not necessary to completely submerge the capsules), then polymerization of resin will be successful.
18. Earlier protocols are confusing in that they report poor sectioning properties of embedding resins if they are polymerized in the presence of water vapor (such as is produced when the water load is heated) [59–61]. However, sealing BEEM capsules with Parafilm appears to prevent moisture from interfering with the polymerization process. In fact, blocks are often immersed in water to make the microwave exposure more uniform. If there is a problem with using Parafilm or submerging in water, then the resin can be polymerized in regular Eppendorf tubes (1.5 ml or 0.5 ml sizes). Eppendorf tubes can be used for LR White or epoxy resin embedding, special sealing

precautions are not required and resin polymerizes even when tubes are only half-filled. Even small amounts of resin can be polymerized. To ensure uniform exposure to microwaves the tubes are partially immersed in water.

19. If the microwave processor has a fitted temperature probe (usually a thermocouple) then it can be used to monitor the liquid temperature around the specimens. However, placing the probe in a blank vial containing the solutions to which the specimen is exposed is recommended. It is possible to obtain specimen baskets with porous sides and/or base that can be placed in shallow dishes for bulk processing of multiple specimens. If this system is used, then the temperature probe is placed away from the specimens, by immersed in the processing liquid. There is still some controversy about the utility of temperature probes in the microwave processor. It is thought that the probe, which is metal, may produce localized heating by attracting microwaves and may not be an accurate indicator of temperature.
20. LR White polymerization requires only a 45 min exposure to microwaves at 100 % power.
21. Although the above protocol suggests using the microwave processor set at 100 % power for polymerization, resins will polymerize using lower power irradiation. Cavusoglu et al. [50] suggest using a preliminary exposure at 100 % power but then switching to reduced power for the remainder of the polymerization time. Polymerization experiments are very simple to perform so new users are encouraged to experiment with different conditions.
22. *SAFETY!* Irradiated materials in the microwave processor can get very hot. Polymerized resin blocks will be immersed in water and may reach temperatures close to its boiling point. Remove the tray from the chamber carefully. Also, toxic vapor emitting from fixatives and other agents may be generated and the risk of inhalation should be considered. An overview of safety considerations when operating a microwave processor has been published elsewhere [62].
23. The advantages of storing mixed resins that do not contain catalyst include convenience and reproducibility. The formulation can be tested and then used when needed if large batches of resin are prepared and frozen down in small aliquots. The absence of catalyst will ensure that the first aliquot will be the same as the final aliquot used. There will not be rapid polymerization of resin occurring during storage, as with storage of prepared resins containing catalyst.

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