In the past few years my laboratory has been exploring alternatives to the conventional lengthy procedures used to fix and embed cells for cellular electron microscopy (microscopy of whole cells and tissues). We start with cells frozen by high pressure freezing, then dehydrate and stabilize the ultrastructure by freeze substitution (FS). We have reduced the time for FS from several days to a few hours. Resin infiltration and embedding is done in 2-3 hours with no special equipment. We also have a new method for on-section immunolabeling by doing FS with uranyl acetate in acetone and embedding in LR White.

**FREEZE SUBSTITUTION.** As described in McDonald and Webb [1] we now do FS using simple, inexpensive equipment instead of a costly automated freeze substitution (AFS) device. Briefly, frozen samples are placed in cryovials containing frozen fixative and placed in a metal block cooled to liquid nitrogen temperature. The metal block is placed in a foam box that is then put on a rotary shaker operating at 100-125 rpm. The samples are warmed up passively over 2-3 hours to room temperature at which point the fixative is rinsed out and the embedding process is begun.

**RESIN INFILTRATION AND POLYMERIZATION.** We used to do quick processing using a microwave oven but wanted rapid procedures that did not require this expensive equipment. We found that microwaves were not actually necessary and also discovered that rapid embedding procedures were not new [2]. Briefly, we do a stepwise increase in epoxy resin:acetone concentrations from 25, 50, & 75%, then 3 times in pure resin for 5-15 minutes each with centrifugation at 2,000 x g for 30 seconds to a minute in between changes. Polymerization is at 100 degrees C for 2 hours. While we can go from live cells to sections in the microscope in one working day, in practice we often freeze, FS, and embed on one day and section and look at the samples the next. Results from a variety of cell and tissue types is shown in recent publications [3,4].

**ON-SECTION IMMUNOLABELING.** Specimens are freeze substituted in 0.2% uranyl acetate in acetone to room temperature, then rapidly embedded as above in LR White which is polymerized at 100 degrees C for 90 minutes. The advantage of not using traditional fixatives is that more antibodies are likely to work at the EM level because the fixatives are not blocking their access to antigens.

Fig. 1: Figure 1 (left) shows chloroplasts from a leaf of white clover (Trifolium repens) prepared by high pressure freezing followed by FS in 2.5 hours and embedding in Epon resin in 3 hours. Bar = 200 nm.

Fig. 2: Figure 2 (right) was prepared by high pressure freezing, FS in 0.2% uranyl acetate in acetone, embedding in LR White, and labeled with 10 nm gold to show actin in the microvilli of C. elegans. Total processing time about 6 hours. Bar = 200 nm.