Transmission Electron Microscopy
Specimen Preparation and Viewing Techniques

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Introduction

Transmission electron microscopy (TEM) is a useful tool for observing extremely small objects with a high resolution. In biology, TEM is used to observe cellular structures such as membranes and organelles.

In order to obtain images using TEM, biological specimens must be meticulously prepared so that they will be able to withstand a high vacuum, intense heat, and yet be thin enough to allow the passing and scattering of electrons necessary to create a usable image. Biological specimens also require special staining techniques in order to increase the specimen's contrast. Preparing a specimen for TEM so that all of these goals are accomplished can be quite challenging.

The purpose of this project was to introduce UWEC students to TEM techniques by processing *Arabidopsis* embryos for TEM viewing. The aforementioned challenges are apparent after analyzing specimens of *Arabidopsis* embryos

Results

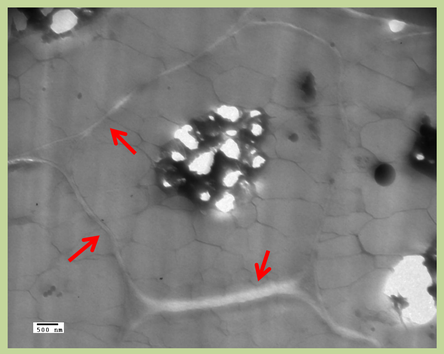


Figure 1: Image represents a condensed and collapsed protoplast. Red arrows indicate cell walls. Magnification is ~4,000x

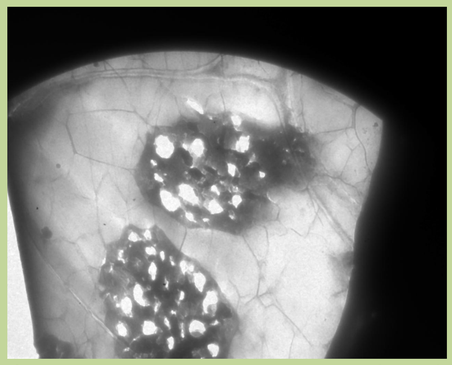


Figure 2: Image represents two condensed and collapsed protoplasts. Magnification is ~6,000x

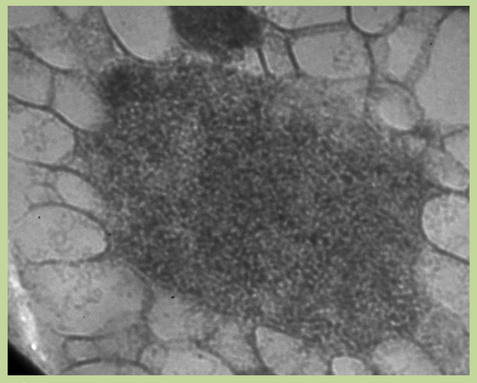


Figure 3: Image represents a condensed protoplast with better infiltration. Magnification is ~8,000x

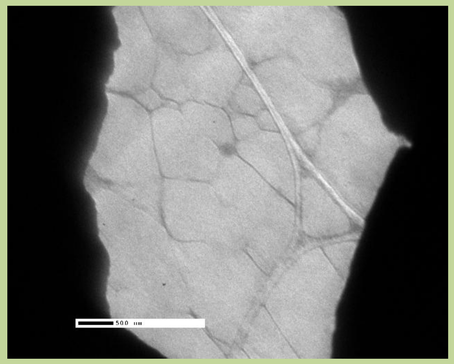


Figure 4: The frustrating effects of specimen charging can be seen it this image. Magnification is ~10,000x

Discussion

According to our images, many problems in specimen preparation altered the cellular structure. The dark regions in figures 1, 2, and 3 are likely to be condensed protoplasts. The holes in the protoplasts in figures 1 and 2 were caused by ice crystal formation and/or poor plastic infiltration. The lack of holes in figure 3 in the protoplast is potentially due to better infiltration of the embedding medium. The "spider web" effects surrounding the protoplasts in all of the figures may be residual cellular components that have remained stuck to the cell walls after protoplast condensation. Another problem we encountered was with anomalies with the TEM itself. Figure 4 demonstrates the frustrating effects of charging. Charging often causes image distortion and a formation of a black "cocoon" around the image.

Future Work

- Modify cell fixation and embedding techniques to eliminate ice crystal damage and infiltration issues respectively.
- Prepare grids prior to collecting sections to prevent melting of specimen.

Methods

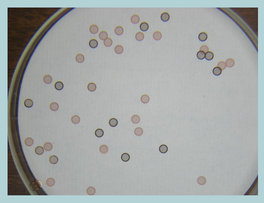
Basic Specimen Preparation for TEM

Biological specimen is fixed chemically to preserve the cellular structure

Specimen is dehydrated chemically and embedded in plastic resin

Embedded specimen is trimmed using a razor blade. Specimen is then mounted in an ultra-microtome (next figure) and finely trimmed

The ultra-microtome is used to cut ultrathin (~60-90 nm) sections of the specimen



The sections are collected onto fine copper mesh grids

The grids are stained to increase the contrast of the specimen

The grids are viewed using the TEM