1

Sample Shrinkage and Radiation Damage of Plastic Sections

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1. INTRODUCTION

Just as fossil insects embalmed in amber are extraordinarily preserved, so are biological samples that have been embedded in plastic for electron microscopy. The success of embedding samples in plastic lies in the astounding resilience of the sections in the electron microscope, albeit after initial changes. The electron microscope image results from projection of the sample density in the direction of the beam, i.e. through the depth of the section, and therefore is independent of physical changes in this direction. In contrast, the basis of electron tomography is the constancy of the physical state of the whole section during the time that different views at incremental tilt angle steps are recorded.

The shrinkage of a plastic section in each dimension, especially the depth, when viewed in the electron microscope, is now a well known phenomenon. Knowledge of the shrinkage behavior of a section of a sample embedded in a particular plastic is of crucial importance when embarking on the electron tomography of the sample. In the last 15 years, the most important advances in electron tomography have been the development of automated methods of recording tilt series and direct imaging onto CCD cameras (Koster *et al.*, 1997; Koster and Barcena, Chapter 4 in this volume). These advances have enabled tremendous savings in labor but also in the total dose experienced by a sample. In this chapter, we review the studies carried out on shrinkage behavior of samples embedded in various resins and we review the protocols that have been followed by the leading proponents of electron tomography.

2. ON RADIATION DAMAGE

Several researchers have written reviews on the effects of the electron beam on biological samples (Egerton *et al.*, 2004; Glaeser and Taylor, 1978; Grubb, 1974; Lamvik, 1991; Reimer, 1989; Stenn and Bahr, 1970). Electron microscope radiation has the primary effect of producing intense ionization in organic materials, which results in the formation of free radicals and ions. This causes bond scission and molecular fragments to be formed. These primary effects occur at all temperatures. At room temperature, the free radicals and molecular fragments can undergo diffusion, and produce cross-