3 Methods for SEM

The surface of an object which is to be investigated by scanning electron microscopy (SEM) must have the following characteristics (Reimer and Pfefferkorn 1977):

- it must be free from foreign particles (e.g. dust, glandular exudates, etc.),
- it must be vacuum stable,
- it must remain stable after exposure to the electron beam,
- it must emit a sufficient number of secondary electrons (see Chap. 1.1.2) and should develop as few surface charges as possible.

Some biological structures fulfill automatically these prerequisites, e.g. the hard exoskeleton of insects or crustaceans, various mineralized structures (teeth, bone, diatom frustules), as well as many structures of plant origin (pollen, wood etc.) In the majority of cases, however, biological objects have to be treated in order that the above requirements may be met. The preparation will differ according to the nature of the object and the method of investigation (imaging with secondary and/or back-scattered electrons, element analysis). Clearly in a book of this nature it is not possible to describe all of the various possibilities which exist; the reader is therefore referred to the more detailed accounts which are given at the end of this introduction.

If the object has already been investigated for TEM purposes and reliable methods on isolation, fixation etc. are at hand, the same method should also be employed for SEM. Should such data not be available, it is always advisable to try to obtain information on the structure and morphology of the object in question using a number of different techniques in order to be certain of the veracity of the SEM results obtained.

Despite having carried out the appropriate preparation procedure, it sometimes happens that the surface is contaminated with large particles or that the desired surface is still occluded within the tissue. This problem can be overcome in some cases with the help of a built-in micromanipulator (Pawley et al. 1975).

The SEM produces images of surfaces which have a strong three-dimensional plasticity. Because of this there is a tendency to interpret the image in real, three-dimensional, terms. One should, however, be aware that the SEM image differs from the true-life object in a number of ways, both as a result of
the method of preparation as well as the way in which the image is constructed. Because of this the production of stereo pairs is an absolute requirement for a detailed three-dimensional analysis of an object. Nevertheless, one has to realize that the recognition of artifacts in SEM is much more difficult than is the case with TEM images.

**Literature**


### 3.1 Conventional Methods of Preparation

#### 3.1.1 Introduction

In the following sections we will present a number of preparatory procedures which are widely used in medicine and biology. These methods have now become standard techniques. Usually the preparation of an object for SEM involves the following steps: