4 Evaluation of Micrographs

4.1 Morphometry

4.1.1 Problems and Solutions

Often a statement about a particular object, made as a result of an electron microscope examination, can only become convincing when backed up with numerical data obtained by image analysis. In addition, a statistical treatment of the measurements made can sometimes give rise to information which is not readily apparent in a qualitative description of the object concerned. Thus, morphometry entails methods of measurement as applied to morphological problems. An important area of morphometry is stereology, which provides the mathematical framework to enable one to create three-dimensional models from measurements carried out on two-dimensional images. Micrographs of sections, freeze-fracture replicas or freeze- or critical point-dried preparations often contain complicated spatial structures to which stereological methods must be applied.

Many particles such as molecules, viruses and bacteria can be characterized on the basis of linear measurements. However, in addition to determinations of length, area, or volume, frequently occurring tasks in morphology are the comparison of corresponding structures in related objects, the description of the development of a particular structure and the analysis of changes in a structure in relation to biochemical or physiological experimentation.

4.1.2 Measurement: Some General Points

Forms such as spheres, ellipsoids, cylinders or cubes are often encountered in bacteria, viruses and molecules and allow area, volume and weight to be calculated from linear measurements. For objects which have a more complicated shape (this applies equally to both small and large structures), the most reliable information is that obtained with the help of serial sectioning, although with the help of stereology it is possible to deduce length, area and volume from objects seen in single sections.

Here we will attempt to give a summary of the most important methods; for more details the reader is referred to the literature at the end of this section.
An important prerequisite in morphometric determinations is the calibration of magnification steps in EM negatives (and, of course, positives). Sometimes true magnifications may vary up to 10% from those values read off the microscope. Again, an internal standard of known size, e.g., latex particles, tobacco mosaic virus or ferritin, which can be included in the micrograph, is of great value.

Another prerequisite in morphometric determinations is a knowledge of all potential sources of artifact as well as of the limits of the particular method of measurement. If an internal standard cannot be included, the extent to which the method of preparation can change the dimensions of the object must be known (e.g., what sort of an effect does spreading have on the length of nucleic acid molecules?). In the morphometry of cells and tissues a factor must be introduced to compensate for potential shrinkages which have occurred during fixing and dehydration. Similarly, the extent or thickness of the coating must be taken into account when dealing with replicas, shadow-cast or SEM specimens. In general, methods of preparation should be standardized as much as possible in order to reduce the number of factors which have to be corrected for.

### 4.1.3 Stereology: General Principles

Stereological methods must be employed when a serial section analysis cannot be carried out and the volume, area, etc., of an object is to be determined. Numerous books are available on this complex subject (see literature list) in which detailed descriptions of the various methods are given.

Before a stereological analysis can be carried out, the micrographs must be checked regarding a number of aspects. Firstly the structures to be investigated must be present in sufficiently large numbers, of uniform size, and they must be easy to recognize in section. Secondly it is assumed that the number of sectional profiles of a structure visible in a particular section is directly related to the total amount of these structures in the object being investigated. It follows then that the sectional profiles must be statistically present in the same number irrespective of the orientation of the section. Whereas this might be true for structures with a homogeneous composition, it is not necessarily so for anisotropic structures. Unfortunately, such structures are often encountered in biological objects, e.g., nerve cells and muscle fibres, and require the application of special stereological methods which have been developed for such purposes.

### 4.1.4 Collection and Evaluation of Data; Statistical Treatments

An \((n-1)\)-dimensional image is presented in a sectional profile of an \(n\)-dimensional structure. Hence a spherical object is seen as a flat profile (in this case a circle), surfaces are represented as lines, and rodlike objects as pointlike structures. In general, the simpler the method used to measure and record these con-