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Soft Part 3D visualization by serial sectioning and computer reconstruction

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Abstract

Recent increases in performance of personal computer hard- and software enabled a variety of 3D graphical applications, such as surface visualizations of biological specimens. This indirectly recalled an old morphological method back to life, the investigation of soft part anatomy by light microscopical serial section analysis. A practical guide covering all procession steps beginning with anesthetization leading to the final goal, 3D visualization of specimens, is provided. Most useful for 3D procession—of particularly small specimens—are ribbon-forming serial resin (= "semithin") sections. A reliable method for achievement of ribbon formation is described in detail for the first time. Contact cement is applied only to the cutting surface of the block, which represents a modification of an old protocol. Details on the materials and tools, such as embedding media (epoxy resins) and knives (Ralph glass or special diamond knife) used and general handling for the entire procedure are given and critically evaluated. 3D procession is explained for the software *AMIRA*®. The major processing steps, from section image capturing until refining of surfaces, are explained. Based on the experience of the author, practical aids that cannot be found in the user's guide of the software or elsewhere for facilitating the process are given. These include preliminary calculation of resolution, calibration and strategies for facilitating the process and improvement of results. The *interpolate* function is emphasized as most useful for completion of *segmentation* and also correction works. Visualization examples are followed by an estimate of work expenditure for graphical processes involved.

Key words: Micromolluscs, methods, histology, serial sectioning, ribbon formation, 3D, reconstruction, visualization

Introduction

Examination of morphological structures by means of serial section analysis reaches back to the 19th century. Originally this was performed by using paraffin for embedding, a method that basically remained unchanged until today. A major improvement to light optic serial section analysis resulted from the invention of transmission electron microscopy (= TEM), which was established as routine method in the 1950s to 1960s. The electron beam used thereby required thinner sections than for light microscopy, which had to be based on a more stable embedding medium. Synthetic resin proved to be the suitable embedding medium for that purpose and soon a variety of different resin kinds together with a new sectioning array (glass knives with floating sections on water) were successfully applied. As a side effect, it turned out that resin sections, if sectioned thicker ("semithin" (!) in contrast to "(ultra-)thin" sections) than for TEM, can be valuable for light microscopical purposes too. In the beginning analyses of resin sections by light microscopy were typically only applied in addition to a main method like TEM investigation. Later on, the merits for light microscopy, compared to conventional methods like paraffin sectioning, were recognized. They particularly regard two fields of application: (1) Because of similarities in texture between the chitin skeleton and the resin, sections

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