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Counting in Nano: Optical Sectioning at the Electron Microscopic Level

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Stereology offers a set of precise tools for the quantitative analysis of 3 dimensional (3D) objects. Most of these tools are based on the analysis of 2D images, sections of the 3D object obtained either by physical or by optical sectioning of either thick slices or whole-mount specimens.
Optical sectioning is gained by the inventive use of the optical lens and aperture system (differential interference contrast, confocal light microscopy, multi-photon microscopy, etc.) to eliminate out-of-focus interference.
Physical sectioning is based on the classical way to produce slices of a sample: cutting them by knife. Optical sections can be produced easier, more efficient, at higher axial resolution and with fewer artifacts. Due to the makeup of a transmission electron microscope, optical sectioning is not possible in the traditional way. Thus, stereological methods involving parallel slices have to rely on the rather inefficient method of physical sectioning.
This becomes apparent when analyzing the application of the disector technique. This technique relies on the occurrence of elements of interest observed between two parallel sections and provides a mean to calculate their cardinality [1]. That the limitations of the physical sectioning are truly a burden to the use of the disector becomes apparent when comparing the number of research articles applying the disector method. In more than 73% of the applications, the optical disector was favoured, and only 1% (3 out of 256) used physical disector on the electron microscopic level. However, help is on its way.

Electron tomography moved into mainstream science around the beginning of the new millennium. While this technique is well researched, the power of its output – a stack of parallel slices at nanometer resolution – is not fully recognized yet (Figure 1). A stack of digitized registered optical images is especially suitable for morphometrical measurements, not in the least because of its increased axial resolution (2-5 nm). The stack is obtained by the tomographic reconstruction of a tilt series: a number of projections of the object at a wide range of angles. For electron tomography, tilts start typically at -60° and are going to +60° with a 1° increment. To obtain useful quantitative information, a (time-consuming) software-rendered visualization of the object is not required, since all information is present in the reconstructed stack of images.

Here, we show the possibility to retrieve quantitative data, estimate important parameters such as volume (Figure 2), surface, length and number by stereology at a resolution of less than 5 nm. The quantitative results were obtained from 3D stacks produced by electron tomography, i.e. without the use of inefficient physical sectioning (one 3D stack of around 200 slices corresponds to one physical section of 300-500 nm).

[1] DC Sterio, *J. Microsc.* 134(1984): 124-136

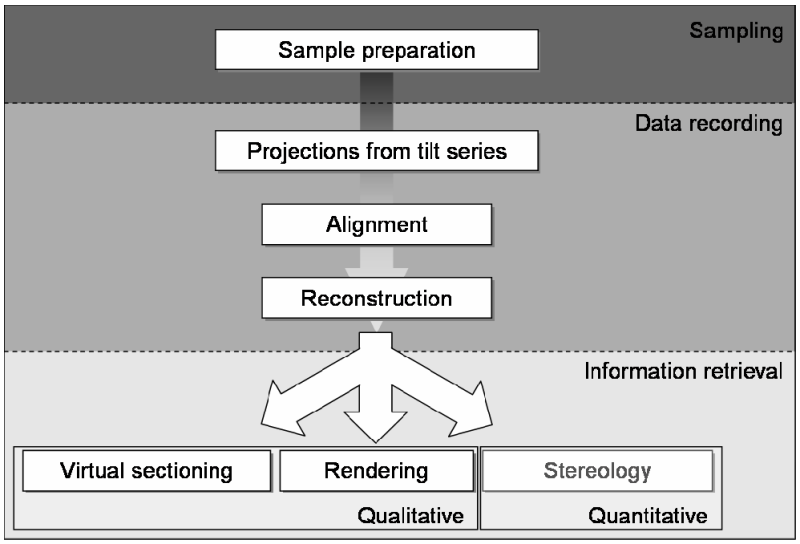
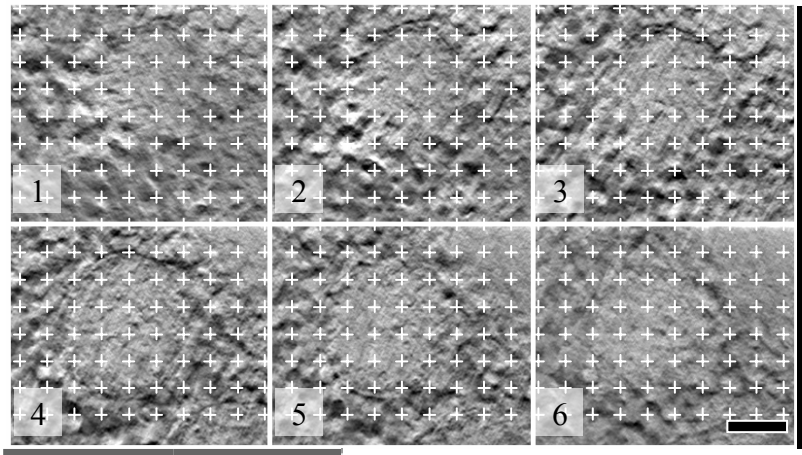
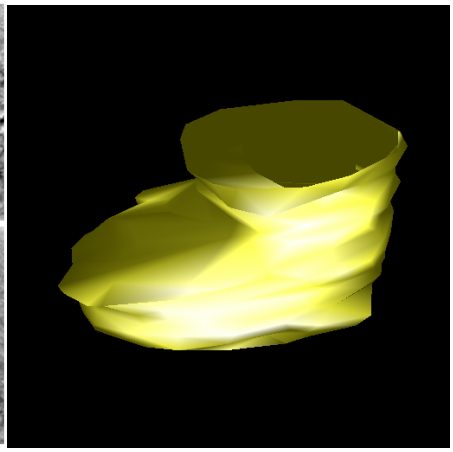


Figure 1. The workflow of electron tomography.
Sampling (the preparation from vital biological material to the retrieval of plastic sections on a TEM grid) happens according to classic electron microscopy protocols. Data recording introduces a rotational relationship between sample and electron source, typical to tomography. The information is routinely interpreted as qualitative. Stereology offers a possibility to quantify the results.





Slice	Points (P)
1 (0 nm)	14
2 (25 nm)	13
3 (50 nm)	20
4 (75 nm)	20
5 (100 nm)	21
6 (125 nm)	15
ΣP	103

Volume estimation formula	ΣP . a(p) . t
Area per point (a(p))	2025 nm^2
Slice distance (t)	25 nm
Volume estimate	**4 802 439 nm^3**
CE(P)	**3.2%**

Figure 2. An example of a volume estimation of a peripheral vesicle in Giardia using the Cavalieri principle. On the left, 6 slices covered by a point grid are shown. The Z distance between two successive slices is 25 nm (bar=100 nm). The table below summarizes the data and the results of the volume calculation. On the right, a 3D rendering of the vesicle is depicted. (rendering performed in Imod; sample preparation by A. Hehl, University of Zürich).

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