doi:10.1017/S1431927610058071
Microsc. Microanal. 16 (Suppl 2), 2010


# TOM$^2$ - A versatile acquisition software designed for 3DEM applications

A. Korinek,* F. Beck,* S. Nickell,* W. Baumeister* and J. M. Plitzko*

* Max-Planck-Institute of Biochemistry, Dept. of Molecular Structural Biology, Am Klopferspitz 18, 82152 Martinsried, Germany

Three-dimensional cryo electron microscopy in life science has become a powerful tool for structural studies. However, structural investigations of frozen hydrated samples require the acquisition of numerous micrographs under strict low-dose conditions. Acquisition of a tilt series for cellular tomography typically requires 50-200 images, recorded in a highly systematic manner. “Single particle” analysis, a powerful tool to determine the 3D structure of large macromolecular complexes such as the 26S proteasome, is even more challenging. Usually acquisition of thousands of high-quality micrographs is necessary to yield a high-resolution structure [1].

Recording of large amounts of high quality data in a solely manual fashion is sometimes impossible, demanding and definitely only possible with a highly trained expert. During acquisition one has to screen and assess the specimen grid at different positions. To keep the optical and imaging conditions constant for each sample position a number of preliminary steps such as auto focusing, beam centering or adjusting the z-height of the stage have to be performed. These tasks are best performed with the help of automated procedures, especially if one aims for better statistics and high-throughput applications. The ultimate goal is the continuous recording of large data sets within days or even weeks on ‘low-maintenance’ microscope systems such as the Tecnai Polara G2 or the Titan Krios (FEI Company, Eindhoven, The Netherlands).

TOM$^2$ is a software package that provides an interface between the human operator and the electron microscope [2]. Complex workflows are translated into microscope operations and designated tasks (see above) are executed in a methodical and completely unsupervised fashion. Based on the ideas of the original TOM toolbox [3] the software uses object orientation programming techniques implemented in MATLAB (The MathWorks, Natick, USA). Real-world objects such as the microscope and its accessories (e.g. CCD, energy filter etc.), micrographs and parts of the acquisition scheme are designed as self-sufficient modules (objects). Each object consists of properties and methods making it an independent “machine” that can interact with other objects. The whole programming can be seen as a collection of objects with defined relationships acting in a highly concerted fashion. The separation between the graphical user interface (GUI) and the underlying microscope-dependent control functions allows a fairly easy adaption of the software to new or different microscope systems. Currently TOM$^2$ supports Tecnai and Titan series microscopes equipped with different types of CCD cameras (e.g. TVIPS, Gatan and FEI Eagle).
The novel acquisition approach of TOM$^2$ subdivides the acquisition into a set of different hierarchical tasks. Instead of a predetermined acquisition scheme, TOM$^2$ allows the user to design his own acquisition protocol by providing simple tools and intuitive GUIs to build up and execute new experiments with the electron microscope. These protocols can be shared and reused by all scientists collaborating on a project, ensuring consistent imaging parameters for all recorded data.

Here we will present the new concept of the TOM$^2$ software package and illustrate its versatility based on examples from single particle investigations and tomography.

References
[1] S. Nickell et al., *FEBS Lett.* 581 (2007) 2751-2756.
[2] A. Korinek et al., *J. Struct. Biol.* submitted.
[3] S. Nickell et al., *J. Struct. Biol.* 149 (2005)
[4] This research was supported by the European Commission in the 7th Framework Program 'PROSPECTS' (HEALTH-F4-2008-201648).

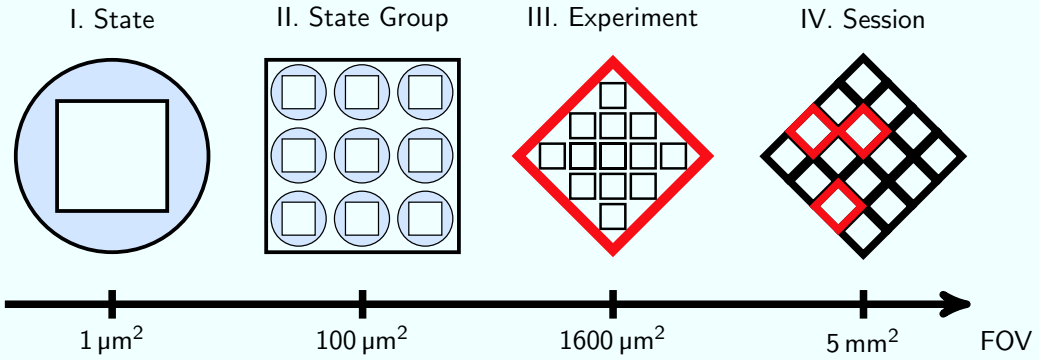


FIG. 1. Hierarchical acquisition scheme of TOM². (I) The "state" represents a set of microscope parameters (e.g. magnification, defocus, CCD exposure time etc.) and an "action" (e.g. acquire, auto focus, tracking etc.). (II) The "state group" combines multiple states at different image beam shift positions to attain low dose conditions during acquisition. (III) The "experiment" combines different state groups, for example: in single particle acquisition a grid mesh is scanned in a meandering fashion using different stage positions; for tomography the tilt angle is iteratively changed after execution of the state group. (IV) The "session" is a collection of several experiments that are acquired sequentially. Each specimen position can be re-centered using a lower-magnification template image. A session can even cover several grids if the microscope is equipped with an automated sample loading mechanism.

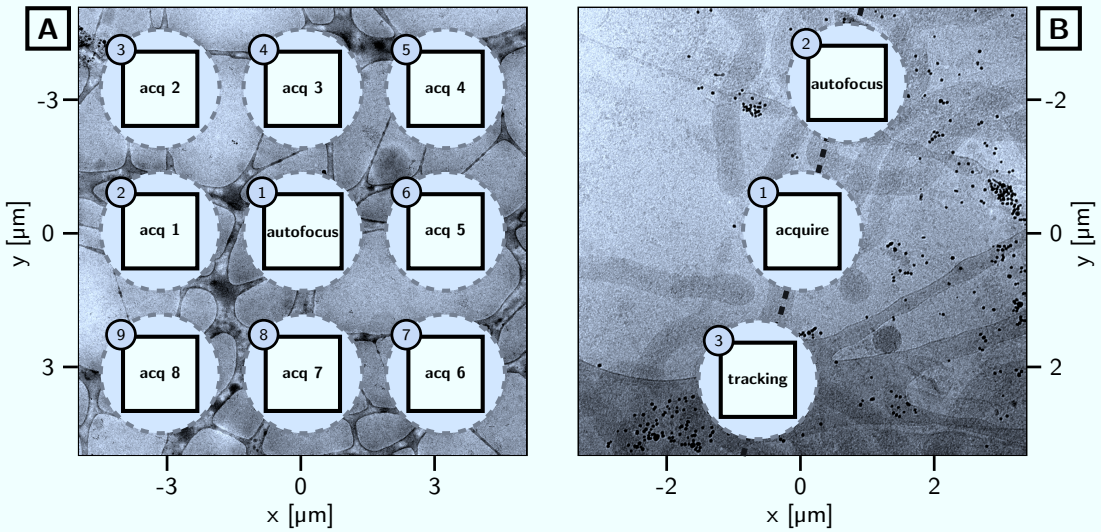


FIG. 2. Example state groups for (A) single particle acquisition, where 8 different acquisition positions and one autofocus position are combined in a group of nine states and (B) tomography, with the standard three-state setup: tracking, autofocusing and exposure.

https://doi.org/10.1017/S1431927610058071 Published online by Cambridge University Press