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TOM software toolbox: acquisition and analysis for electron tomography

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Abstract

Automated data acquisition procedures have changed the perspectives of electron tomography (ET) in a profound manner. Elaborate data acquisition schemes with autotuning functions minimize exposure of the specimen to the electron beam and sophisticated image analysis routines retrieve a maximum of information from noisy data sets. 'TOM software toolbox' integrates established algorithms and new concepts tailored to the special needs of low dose ET. It provides a user-friendly unified platform for all processing steps: acquisition, alignment, reconstruction, and analysis. Designed as a collection of computational procedures it is a complete software solution within a highly flexible framework. TOM represents a new way of working with the electron microscope and can serve as the basis for future high-throughput applications.

Keywords: Electron tomography; Cryo-EM; Automated data acquisition; Reconstruction software

1. Introduction

Electron tomography has a great potential for structural studies, both in biology and in materials science. Unlike other EM techniques it provides three-dimensional images not only of periodic or repetitive objects, but also of structures with unique topologies. In biology it opens a new window into the machinery of cells on different levels of complexity: from isolated macromolecular assemblies and organelles to intact cells. With biological material it is highly desirable to combine the potential of three-dimensional imaging with a close-to-life preservation of the specimen. Cryo-electron tomography, i.e., the application of tomography to vitrified samples, has to cope with the high-sensitivity of such samples to ionising radiation. To avoid that radiation damage erases structural information, the cumulative dose during data collection must be kept subcritical and, consequently, exposure to the beam must be minimized rigorously. Minimal dose data acquisition schemes rely on elaborate computer software, including autotuning functions which correct for mechanical imperfections of the microscope. Information extraction is achieved by means of image-processing tools that are specialized for the analysis of noisy data sets. A number of dedicated software packages are available in the field of biological electron microscopy, providing solutions for electron crystallography and single particle analysis (Aebi et al., 1996; Carragher and Penczek, 2003) (for an updated list see http://3dem.ucsd.edu/Software.htm). In the field of electron tomography, the leading laboratories, the EM manufacturers and providers of accessories such as CCD cameras have developed their own software based on similar acquisition and analysis strategies (Frank et al., 1996; Koster et al., 1997; Kremer et al., 1996). The differences in the software result from adaptations to specific instruments or the specific needs of the samples under

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investigation. Interchange or transfer of programs during recent years has been limited and, as a consequence, software solutions have been rewritten a number of times.

With the advent of a new generation of electron microscopes like the *Tecnai* series (FEI Company, Eindhoven, The Netherlands), it became feasible to access important microscope parameters via a common software standard, the *COM* interface (Compound Object Model, Microsoft, Richmond, USA). Via this interface, external e.g., non-FEI proprietary software is enabled to read and modify microscope parameters such as magnification or defocus. Furthermore, it allows one to call up internal procedures for e.g., lens normalization or to invoke routines such as the *autofocus* function implemented within FEI's tomography package. This 'open machine' concept allows access by other software languages and mathematical programming environments such as *Matlab* (The MathWorks, Natick, USA). Based on the scientific computing platform of *Matlab*, a collection of different routines was developed which not only include the procedures for acquisition of tomographic tilt series, but also the alignment, three-dimensional reconstruction and subsequent three-dimensional analysis of tomograms. By combining algorithms from the established *EM* software package (Hegerl, 1996) with new concepts and routines tailored to the needs of low dose tomography, a new software package was created: the 'TOM toolbox.'

2. Overview and software philosophy

The TOM toolbox is structured thematically according to the steps involved in the acquisition and reconstruction of tomographic data sets: *Acquisition*, *Reconstruction*, *Analysis*, *Display*, *Filtering and Transformation*, *Spatial Transformation*, *Average*, *Geometrical shapes*, *Input/Output*, *Miscellaneous*, and *Utilities*. Data, such as images or volumes, can be loaded into the *Matlab* memory environment and then processed by typing function names in a command window style. Most of these functions are equipped with a graphical user interface (*GUI*), thereby allowing user-friendly access to the program's functionality. For complex tasks like the acquisition of a tomographic tilt series, the user can modify parameters directly through the exclusive use of these interfaces. Although most of the functions are used in a typical programming style, they can be adapted very easily and implemented in procedures, so-called "*M-files*" of the *Matlab* language. The whole 'look and feel,' including the way of documentation is compatible to the *Matlab* style and therefore straightforward for the experienced user and easy to learn for the beginner. Therefore, the toolbox is simple enough to be used routinely by all laboratory personnel without a long learning curve, and sufficiently expandable to allow the advanced user to incorporate new procedures that enable him to address specific problems. The TOM toolbox provides dozens of basic and advanced image processing procedures that are optimised especially for the field of electron tomography. These routines allow a direct manipulation of multi-dimensional data sets, and extend the capabilities already provided by *Matlab* and its various toolboxes including the *Image Processing Toolbox*.

3. Applications

The TOM toolbox is organized as a collection of modular routines (Fig. 1). Therefore, the execution of a particular task such as the acquisition of a tilt series is done by a combination of single operations. A detailed description of the basic sequence in obtaining tomographic data together with the *modus operandi* for its algorithmic realization will be given in the following paragraphs.

3.1. Acquisition of tilt series

The main rationale in data acquisition with biological material is the minimization of the exposure of the sample to the electron beam. To avoid radiation damage, the

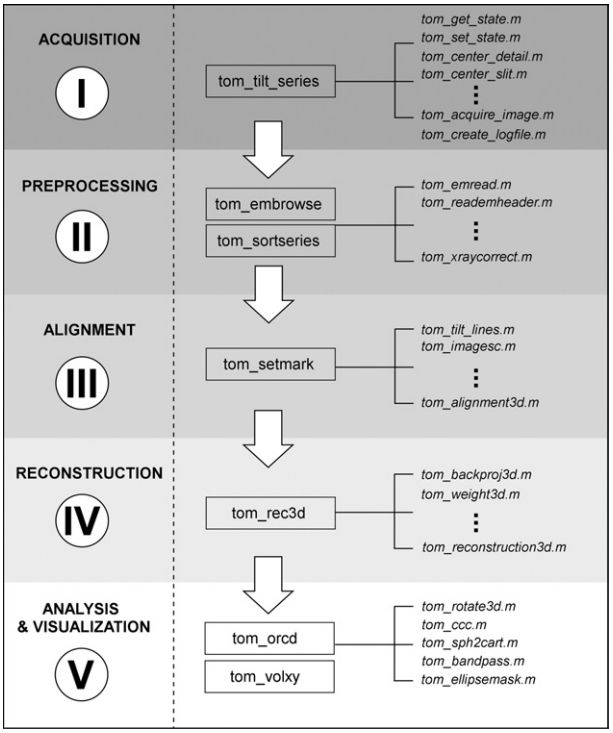

Fig. 1. The TOM toolbox integrates all relevant steps in tomographic data processing within one platform. The current applications range from data acquisition to preprocessing and data evaluation. After alignment and reconstruction, the tomograms can be visualized. In addition, three-dimensional analysis and postprocessing routines are implemented, providing e.g., filters, rotations or various spatial transformations. The higher-level procedures are programmed in a modular way indicated by the right-hand column of subroutines.

cumulative electron dose should not exceed 50–80 e/A^2. As a consequence of fractionating this dose over a large number (>100) of projection images, the signal-to-noise ratio (*SNR*) of each projection image is very low. Moreover, since the mechanical accuracy of the goniometer, operated in conjunction with cryoholders is only in the micrometer range with most electron microscopes, spatial shifts in *x*, *y*, and *z*-direction must be compensated electronically by corresponding image shifts. Also other parameters (spotsize, beam intensity, and exposure time) need to be adjusted in the course of recording tilt series. This is done during data acquisition by a *tracking* task, allowing a translational re-centering of the sample, and a *focus* task, correcting for the defocus change arising from movements in the *z*-direction. To protect the sample, these correcting tasks are done with an experimental setup different from the final acquisition situation. Various parameters including the position of the imaged area, exposure time, or binning of the CCD camera can be adapted for each specific situation, which is reflected by distinct states. That way a feedback loop is created to adapt online the settings of the microscope. Therefore *tom_tilt_series* allows direct access to all essential parameters to define the appropriate imaging conditions. They are organized in substructures of a *state* variable which include all the different components of the microscope, e.g., the electron optical settings, the energy filter and the CCD camera. All parameters can be retrieved and sent to the electron microscope. Additionally, an *Image Processing* substructure defines the parameters for a postprocessing step such as filtering or smoothing of the image borders, which can be applied to an individual image in the particular state. In the current implementation of *tom_tilt_series*, there are four different states: *Search*, *Tracking*, *Focus and Acquisition*. These can be accessed interactively by a graphical user interface which is shown in Fig. 2. The parameters are grouped horizontally, indicated by different colours

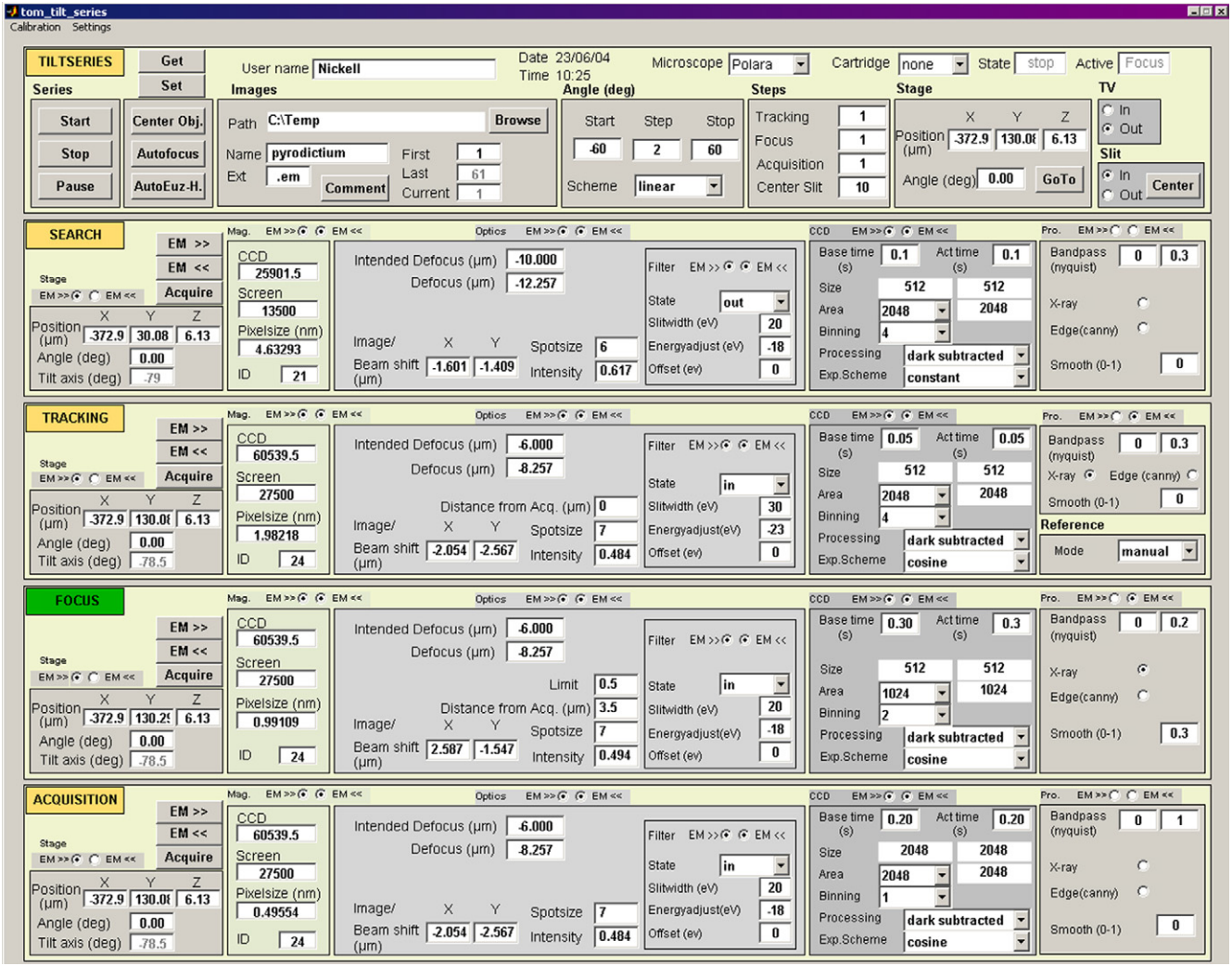

Fig. 2. Graphical user interface (GUI) for the automated acquisition of tomographic tilt series. The four states *Search*, *Tracking*, *Focus*, and *Acquisition* reflect different imaging conditions of the electron microscope required for correction tasks. Arranged horizontally, the parameters are grouped according to different colours, which also reflect the underlying data hierarchy.

reflecting the underlying data organisation, yet form one particular state. Parameters defining the tilt series are collected in another data structure; here, the angular range, increment, tilting scheme (Saxton et al., 1984) and auxiliary variables such as the path or the file names are stored. After setting all states, recording of the tilt series can be started and proceeds automatically through the scheme of tilting, tracking the sample position, focusing, energy slit centering and image acquisition. Currently, two procedures provided by the FEI tomography package are used in combination with the setup described: the *autofocus* routine and the *autoeucentric height* routine. The former is invoked during the tilt series following the setting of all parameters within the *tom_tilt_ series* user interface. The latter can be used prior to data acquisition to adjust the *z*-height of the specimen minimizing the lateral shifts induced by tilting the specimen. During the acquisition process, a log file is generated, tracing all important parameters in a protocol-like manner. In parallel, the *GUI* is updated after each step to provide the user with information about the current microscope settings. A *pause* button allows the user to interrupt the program flow. Especially at high-tilt angles, where problems with autofocusing or autotracking due to the increased thickness are notorious, one is able to readjust e.g., the image processing options manually. Therefore, pausing the acquisition process helps and in some cases enables the operator to obtain optimised tomographic data sets.

3.2. Alignment of tilt series

During the acquisition of a tomographic tilt series, the displacements caused by not perfectly eucentric goniometers are compensated over a wide range. Nevertheless, this compensation is usually insufficient—small residual image shifts in the range of several nanometers make it necessary to align the projections a posteriori more accurately with respect to a common coordinate

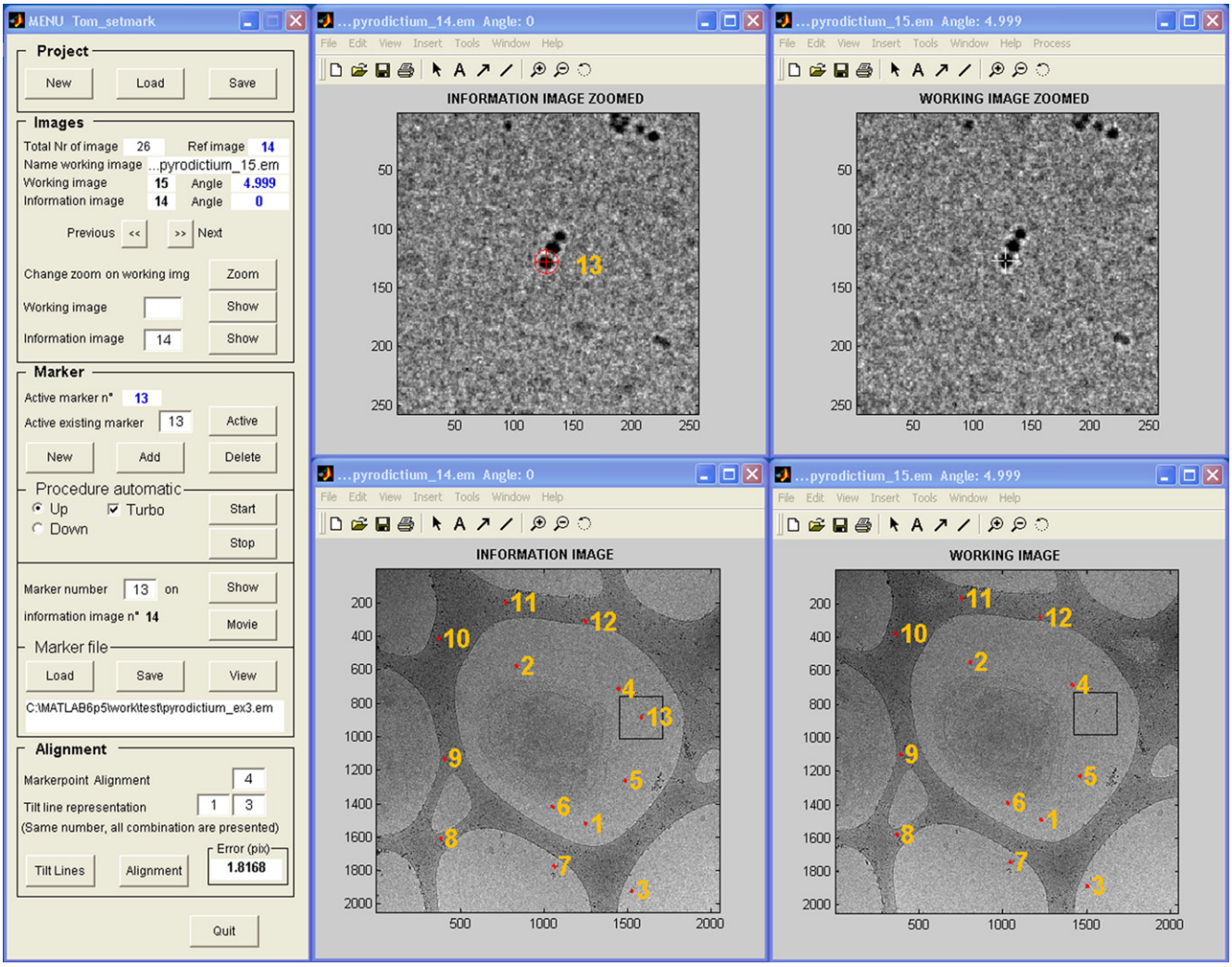

Fig. 3. Interactive alignment tool (*tom_setmark*) for the selection of fiducial marker points in single tomographic projections. To enable an accurate selection of marker coordinates, two enlarged image areas are provided (top images). Images arranged on the left side show the previous position of the marker.

system for the subsequent 3D reconstruction. There are two principal ways to solve this problem: the individual micrographs can be aligned using the entire grey value information, e.g., using cross-correlation based algorithms (Liu et al., 1995), or by determining the common coordinate system from several fiducial markers (Lawrence, 1992). Within the TOM toolbox, the latter approach is implemented since it works reliably for projections having a very low SNR. Electron-dense markers are often present in the form of gold beads (typical diameter 5–20 nm), which are added to the sample solution immediately prior to cryo-fixation. Ideally, these beads are homogeneously spread over the whole field of observation, and are clearly visible in all projections of the sample. Assuming a spherical shape of the markers, the positions are extracted in all projection images of the complete tilt series. Knowledge of these marker positions allows calculation of a common three-dimensional coordinate system and subsequently, a determination of the tilt axis direction. Implemented in a very user-friendly way, TOM provides an interactive tool for marker alignment: *tom_setmark*. Equipped with a *GUI* (Fig. 3), this procedure allows the selection of a marker point simply by a click with the computer's mouse. A *zoom* option is included to enlarge particular regions of the image, representing a very convenient way of editing the marker points. If an initial marker has been identified and aligned in all projections, a simple prediction model is used to estimate the shifts of all subsequent markers. The image's region of interest is thereby re-centered to the expected position allowing an even faster selection procedure. Marker points move along straight lines in a tomographic tilt series. This knowledge is used in *tom_setmark* not only to monitor the marker movement interactively but also to calculate the projection shifts. This is done by fitting the difference vectors of all markers to straight lines perpendicular to the tilt axis. A root-mean-square deviation of this procedure is used as a quality criterion for the alignment. The actual implementation of the alignment procedure corrects for the lateral shifts of the micrographs and the tilt axis as implemented previously in the *EM* program (Hegerl, 1996).

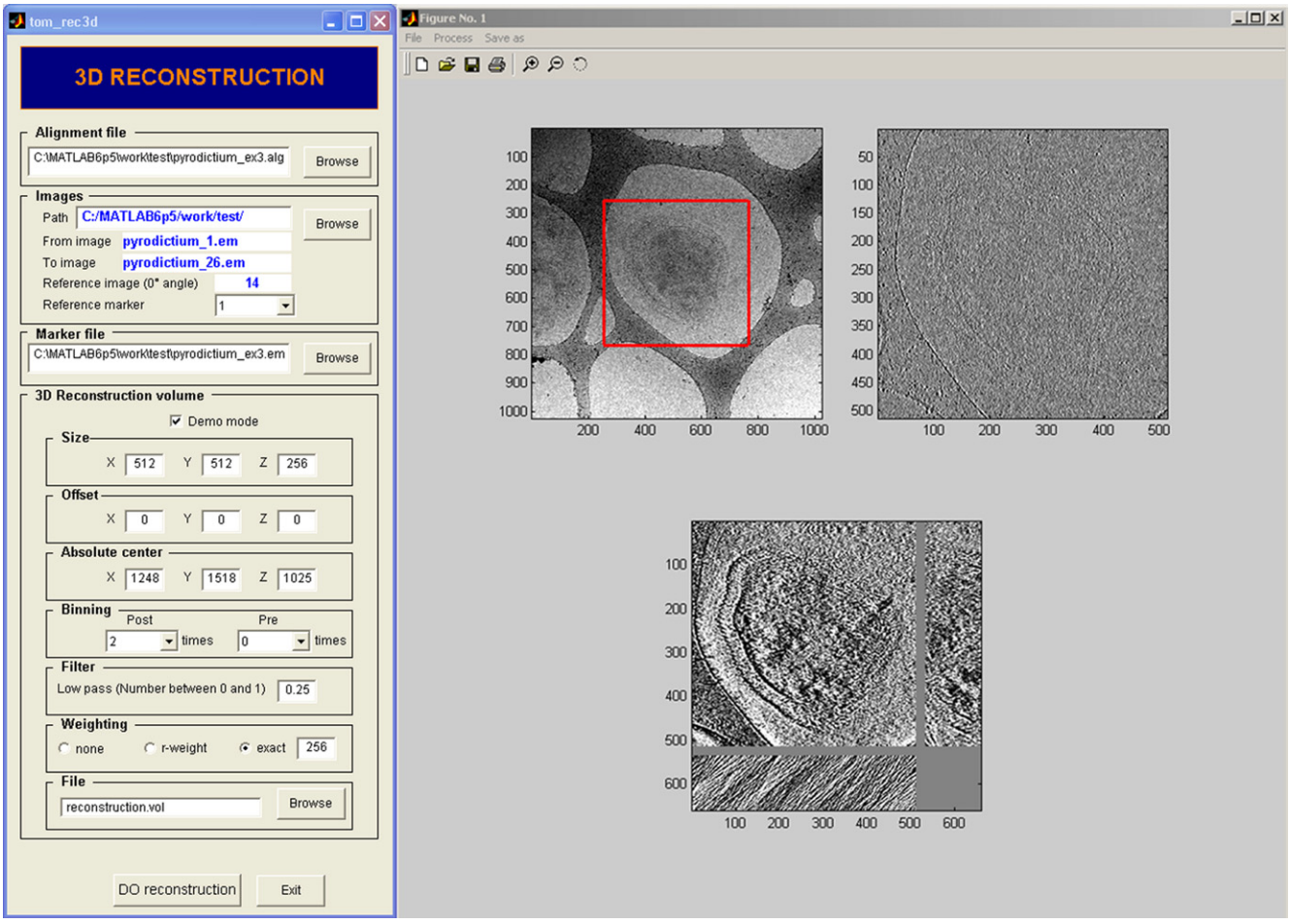

Fig. 4. User interface for the three-dimensional reconstruction procedure. A 'demo' mode allows online inspection of the current processing steps. The upper left image shows the original projection, with a red rectangle indicating the size and position of the reconstructed region. Next to this, the aligned and shifted area of the projection is displayed. The lower image shows the central slice of the reconstructed tomogram in the *xy*-, *yz*-, and *xz*-directions.

3.3. Reconstruction

Following successful alignment, the three-dimensional reconstructed volume can be generated using weighted backprojection. For this purpose, the projection images have to be shifted to a common origin defined from the calculated marker model. Overemphasis of low frequencies in Fourier space is avoided by using a weighting scheme prior to the reconstruction. In the reconstruction procedure implemented in TOM, two different schemes in Fourier space are included: an analytical, frequency-dependent weighting, and a weighting function for arbitrary projection geometries (Radermacher, 1988). Subsequently, the aligned and weighted projections are backprojected into a three-dimensional volume using trilinear interpolation.

All processing steps mentioned thus far are independent procedures. They can be used in a combined way via a *GUI*, where all essential parameters are accessible directly (Fig. 4). This user interface includes a demonstration mode which allows an online inspection of all computational steps during the reconstruction process. For a tomographic reconstruction of sub-volumes with e.g., a higher specified resolution, a different routine called *tom_particles* can be used. This procedure allows the extraction of features, like macromolecular structures, which can be selected in an overview representation, followed by a reconstruction of smaller volumes comprising only the particle and its neighbourhood.

3.4. Visualization

For further analysis, TOM provides some basic graphical representation procedures including *tom_volxy* or *tom_intervol*, which enables the user to browse the reconstructed tomograms interactively (Fig. 5). This can be done easily by moving a slider indicating the current position (z) within the volume. The accompanying histogram can be used to enhance the contrast and brightness of the displayed image. Details can be magnified or demagnified with a 'zoom' button. These tools are implemented in two different ways. One is

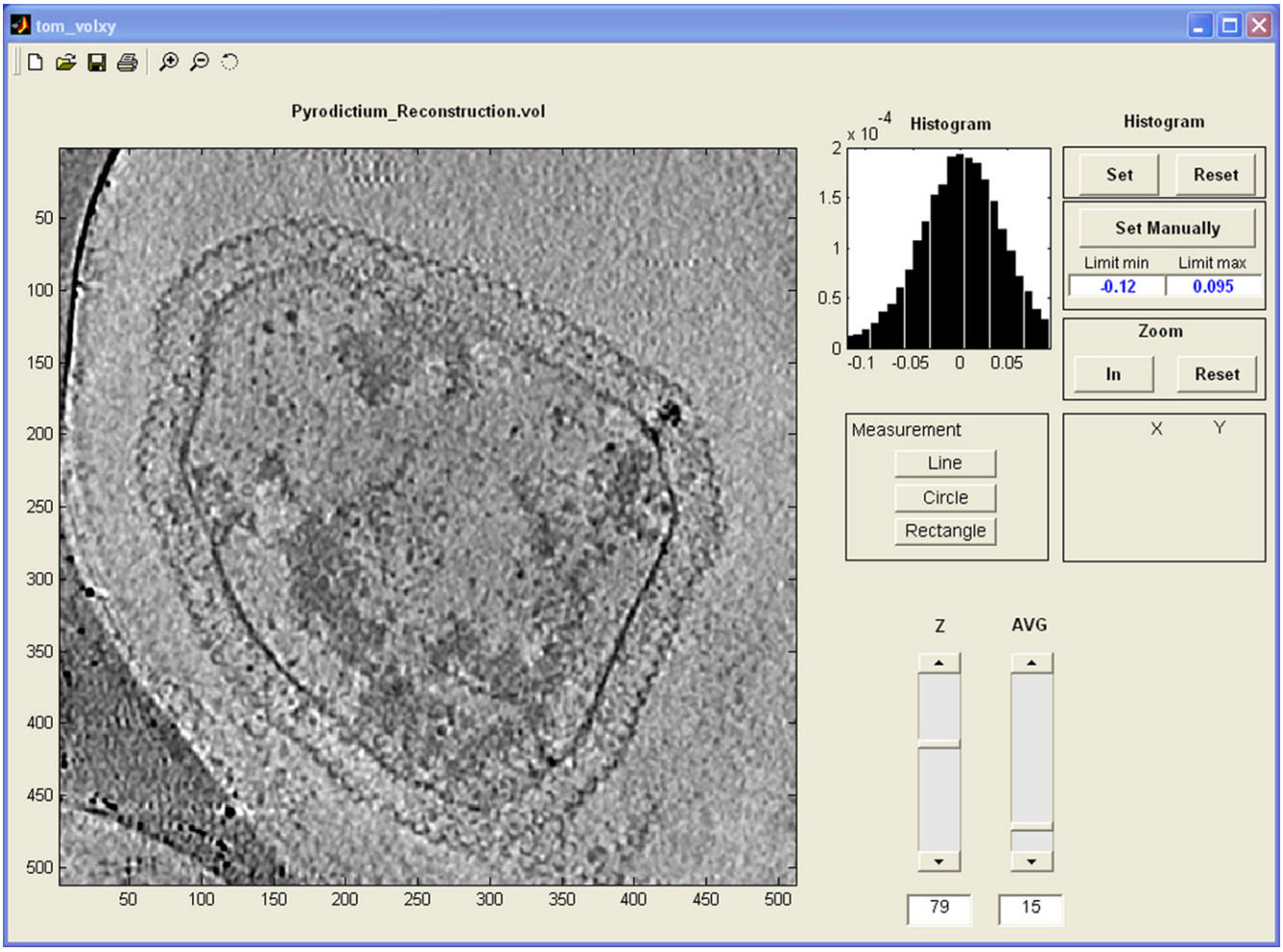

Fig. 5. The volume browser *tom_volxy* allows the visualization of single slices of the reconstructed volume. Averaging several slices in the z-direction improves the signal-noise-ratio of the displayed xy-slice. The contrast can be altered by an interactive adjustment of the histogram.

memory-based and consequently fast; however, it is limited to the size of the displayed volume. The other one includes a fast-reading routine which runs directly from the hard disk, allowing the inspection of huge datasets, albeit with slower performance. This is a considerable drawback if data are loaded via a slow network connection.

4. Technical aspects

4.1. Requirements

Requisite for the use of the TOM toolbox is *Matlab* (Version 6.5.1 or higher) and the *Image Processing* toolbox (Version 4.1 or higher, The MathWorks, USA). The existing acquisition software is written exclusively for a *Tecnai* electron microscope (FEI Company, Eindhoven, The Netherlands) equipped with a Gatan *Imaging Filter 2002* (Gatan, Pleasanton, USA). To make use of the data acquisition within the TOM toolbox, *Matlab* must be installed at the microscope computer in addition to FEI's tomography package, and Gatan's *Digital Micrograph* and *Filter Control*. All other procedures of the TOM toolbox are highly cross-platform compatible and do not require any supplementary software. The TOM toolbox has been tested for *Linux*, *Irix* (SGI), *OsX* (Apple), and *Windows* (Microsoft).

4.2. Data formats and structure

The data format of choice within the TOM toolbox is the so called '*EM*' format (Hegerl, 1996), which is basically a 512 byte header followed by raw data in *xyz*-directions, where *x* is the fastest variable. The header information is stored in a variables substructure; for example, if an '*EM*'-file is loaded to a variable *In*, the header is stored in *In.Header*, whereas *In.Value* carries the raw data information corresponding to the *Matlab* matrix convention. TOM is already equipped with data-reading routines for other formats ['*MRC*'-format (Crowther et al., 1996) and '*Spider*'-format (Frank et al., 1996)], but full functionality requires conversion to '*EM*'-format style.

4.3. Implementation of algorithms

All computational routines were designed and implemented in a transparent way, thereby allowing a rapid understanding of the underlying algorithm for the advanced user. The programming philosophy of TOM toolbox is to use pre-existing functions provided by *Matlab* and the *Image processing toolbox* to the greatest possible extent. It was found that these functions are well tested and already highly optimised in terms of computing performance and memory requirements. An example is the function to perform a fast Fourier transformation which has a comparable execution time to the corresponding function in the *EM* package. Some computationally expensive routines, like the three-dimensional backprojection algorithm were implemented in *C* using an interface provided by *Matlab* to call external programs (generally supported are *C* and *Fortran* programs and *Java* classes). When compiled on the specific platforms, these functions appear in the *Matlab* environment according to all other built-in routines. In this way, TOM toolbox can handle even large reconstruction volumes without any loss in computing performance.

4.4. Documentation

Updates of the TOM documentation are available via a web page hosted at the Max Planck Institute of Biochemistry in Martinsried, Germany. At the TOM homepage (www.biochem.mpg.de/tom), documentation is structured thematically, whereas the acquisition part, due to its complex architecture, is organized into several substructures. The download of a sample data set from this homepage is also possible to allow a 'quick-start' for beginners. References to mathematically advanced algorithms are cited within the M-files to facilitate in-depth understanding of the particular problem.

5. Discussion and outlook

Despite, the fact that *Matlab* is a commercial product provided by the MathWorks, which makes one dependent on a single software provider for future developments, it is an established, well tested program widely spread throughout science and industry and in many different disciplines. Especially in the field of electron tomography, recent developments integrate the processing and visualization of three-dimensional data and tomographic reconstruction routines. Moreover, the latest version of *Matlab* supports the new architecture of 64-bit processors, allowing an extended allocation of memory important for computational tasks of large data sets.

Designed for an electron microscope of the *Tecnai* series, TOM toolbox currently supports only one type of instrument equipped with a specific CCD camera. Nevertheless, the software can be easily adapted to any microscope and camera type by modifying only several routines, provided that an access via the *COM* interface can be established.

Approaches to the structural analysis of biomolecular systems have to be optimised for various types of specimens. For studying homogeneous preparations of macromolecular structures, the now widely established 'single molecule' approach (Frank, 2002) is most effective, and great strides have been made in automating

data acquisition and analysis to the extent that 'high-throughput' is a realistic goal (Carragher et al., 2004). For structurally more variable macromolecular samples or for studying macromolecules in their functional environment, i.e., in organelles or cells, electron tomography, ideally in a non-invasive mode and applied to a close-to-life state of the system is the method of choice (Baumeister and Steven, 2000). Due to differences in instrumentation and expertise, several software platforms that differ in performance and flexibility have been developed. TOM takes a different approach: it presents a flexible system, which not only covers data analysis but also integrates the acquisition process under one main platform. While the unification of the entire workflow via TOM is the basis for future high-throughput applications, TOM also represents a new way of working with the electron microscope. Traditionally, most decisive steps in aligning and adjusting electron microscopes are complex and time-consuming, requiring a specialised and experienced operator. In principle, such a task is better suited for a computer than a human operator. Automated algorithms can take over the elaborate sequence of repetitive alignment steps. Thus, optimised imaging conditions can be attained routinely, so that even novices can take full advantage of modern, high performance electron microscopes.

Furthermore, image processing routines which are normally applied a posteriori, for example corrections for geometric image distortions and wave aberrations, can be easily developed and implemented for online usage to improve and assess the image quality. The exact defocus can be determined by analysis of Thon rings and fitting the analytical contrast transfer function (Meyer et al., 2002). Sophisticated pattern analysis tools like template matching (Potter and Zhu, 2004) can be used to extract features invisible to the human eye and to retrieve a maximum of information. This, in turn, provides a basis for refining experimental parameters and for selecting volumes of interest through a preview option. Especially in cellular tomography, where many structures with unique topologies are examined and virtually no identical objects exist, this could be advantageous as well as being a significant impetus for the development of high-throughput applications. To foster the development of new approaches in electron microscopy, the TOM toolbox is provided as a project with full access to source code and procedures. It is also intended as an open forum for the exchange of algorithms, allowing a transparent comparison of different methods in the field of materials science and biological structural analysis. After publication, an algorithm can be provided to a broad audience via the toolbox, whereas all rights and credits still belong to the developers. Contributions are currently accepted at the MPI of Biochemistry, Martinsried, Germany where TOM toolbox is available to anybody interested in the field of electron tomography. The program is provided at no cost to users without commercial interests (www.biochem.mpg.de/tom).

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