

Modular software platform for low-dose electron microscopy and tomography

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Key words. Automation, cryoEM, data acquisition, software.

Summary

Transmission electron microscopy imaging protocols required by structural scientists vary widely and can be laborious without tailor-made applications. We present here the JEOL AUTOMATED MICROSCOPY EXPERT SYSTEM (JAMES) API INTEGRATOR, a programming library for computer control of transmission electron microscopy operations and equipment. JAMES has been implemented on JEOL microscopes with Gatan CCDs but is designed to be modular so it can be adapted to run on different microscopes and detectors. We have used the JAMES API INTEGRATOR to develop two applications for low-dose digital imaging: JAMES imaging application and the MR T tomographic imaging application. Both applications have been widely used within our NCCR-supported Center for routine data collection and are now made available for public download.

Introduction

A modern transmission electron microscope can be employed in a number of different ways. It can be used to observe specimens ranging in scale from complete cells down to individual atoms from biological or non-biological origins. The techniques used to visualize structures at these different scales require a diverse combination of microscope accessories and detectors, often from several different manufacturers. Frequently, many of these components require computer control to be used effectively. Unfortunately, the software to run these components is rarely shared between companies, resulting in partially integrated components, supporting only

limited experimental tasks. In particular we found no available solution for low-dose imaging that integrated software control of our Gatan CCD cameras with the FASTEM software package (Fukushima *et al.*, 2000) that controls our JEOL microscopes. To meet our needs but also establish a more general solution, we found it necessary to develop a package that handles communication between electron microscope components and provides researchers with a single interface to their equipment for software engineering.

We have chosen to develop a modular software platform called JEOL AUTOMATED MICROSCOPY EXPERT SYSTEM (JAMES), which is a programming library and set of prototype applications to assist the skilled microscopist with routine microscopy tasks on JEOL microscopes running FASTEM. JAMES does not attempt to oversimplify the technique, but instead allows the operator to stay in control of most of the decisions throughout the course of a microscopy session. This approach has allowed our operators to quickly prototype new image collection protocols and optimize data quality without having to spend time working with software packages from multiple manufacturers.

Materials and methods

Hardware

All development and testing were carried out on a JEM-2010F and a JEM-3000SFF (JEOL USA, Peabody, MA), 200- and 300-keV transmission electron microscopes. These microscopes are under computer control managed by JEOL's FASTEM 3.00 application (Fukushima *et al.*, 2000). FASTEM is a client/server application, enabling the microscopist to use a remote FASTEM client as a graphical microscope control

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interface that delegates directives across the network to the FASTEM server connected directly to the microscope.

Both microscopes are connected to Gatan Ultrascan 4000 slow-scan CCD cameras (US4000) (Mooney, 2007) (Gatan, Pleasanton, CA). The US4000 is a Peltier-cooled 4096×4096 camera with a four-port readout. Camera control is managed by Gatan's DIGITAL MICROGRAPH (DM) application (GMS 1.6) (Mitchell & Schaffer, 2005), which runs on a workstation proximal to the microscope.

Programming languages and libraries

All C++ programming was carried out with VISUAL STUDIO.NET (Microsoft Corp., Redmond, WA). PYTHON code was written to be compatible with the PYTHON 2.3 specification and later validated with PYTHON 2.4 (<http://www.python.org>). Access to the C++ libraries from PYTHON was established with the BOOST TOOLKIT for C++ (<http://www.boost.org>). Our JAMES-enabled workstations run on either WINDOWS 2000 or WINDOWS XP (Microsoft Corp.).

Results and discussion

JAMES API INTEGRATOR

Software architecture. To implement microscope control software in a general way, we have developed the JAMES APPLICATION PROGRAMMING INTERFACE (API) INTEGRATOR. The JAMES API INTEGRATOR is a programming library that scientists can use to develop individualized microscopy applications. The JAMES API INTEGRATOR unifies the microscope and its disparate accessories in one library so that application developers do not need to learn vendor-specific APIs for each component they wish to control. By decoupling all of the microscope and camera control from the highest layer of the programming interface, we have isolated the application programmer from all of the details of a particular vendor's microscope or camera. Yet the software has been designed to be modular and replaceable; cameras can be added or replaced, and, in principle, the microscope itself could be replaced by a newer microscope of a different brand or model. Fig. 1 illustrates the overall layout of software in the JAMES API INTEGRATOR.

FASTEM interface. JEOL offers a client/server system called FASTEM for controlling the microscope. The FASTEM server is connected directly to the microscope and can receive communication from multiple FASTEM clients. JEOL provides the FASTEM server and a basic FASTEM client API that was written in C (<http://www.jeolusa.com>). We wrapped this FASTEM client API in C++ to abstract some of the existing libraries and make them callable from PYTHON (Fig. 1). PYTHON is a popular scripting language, which has been widely adopted by the open source community and especially by the structural biology community (Hohn *et al.*, 2007). Exporting the C++ API to a PYTHON API was carried out with the BOOST toolkit.

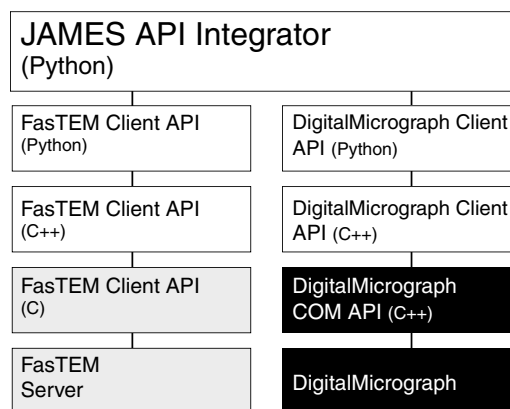


Fig. 1. JAMES architecture. JAMES unifies the software from JEOL and Gatan into a single PYTHON API. This is achieved by writing C++ modules that communicate with FASTEM and DM and interface with PYTHON using BOOST.

DIGITAL MICROGRAPH INTERFACE. DM provides a rich scripting language called DMScript. It is similar to C++ and can be used to control the operation of the CCD camera. Based on a template provided by Gatan, we implemented our DM client API in C++. It communicates directly to DM by sending DMScript commands and allows us to make DM functions callable from PYTHON. We again used the BOOST toolkit for this purpose (Fig. 1).

JAMES API INTEGRATOR layer. The JAMES API INTEGRATOR unifies the underlying layers described above and makes the interface to the microscope and its accessories visible to the scientists in one simplified API. For example, the scientist uses the JAMES API INTEGRATOR to interrogate the microscope at runtime to determine which detectors (i.e. photographic film camera or CCD camera) are available, and then selects which detector to use. The software will appropriately delegate function calls to the chosen detector. The usefulness of this library is made evident by the two imaging applications described below.

JAMES: a single-particle application

Transmission electron microscopy of ice-embedded biological specimens (electron cryomicroscopy or cryoEM) is a technique by which large numbers of macromolecules in different orientations are imaged and averaged together to produce a 3D reconstruction. Current data sets require several thousands to hundreds of thousands of particle images. The requirement for so many images has prompted a number of groups to propose and implement automation of data collection to reduce the amount of time that an operator must spend in the highly repetitive task of image acquisition (Potter *et al.*, 1999; Stagg *et al.*, 2006; Zhang *et al.*, 2003).

The JAMES single-particle application (Fig. 2) developed with the JAMES API INTEGRATOR is our approach towards automation in the JEOL microscope. The application supports low-dose

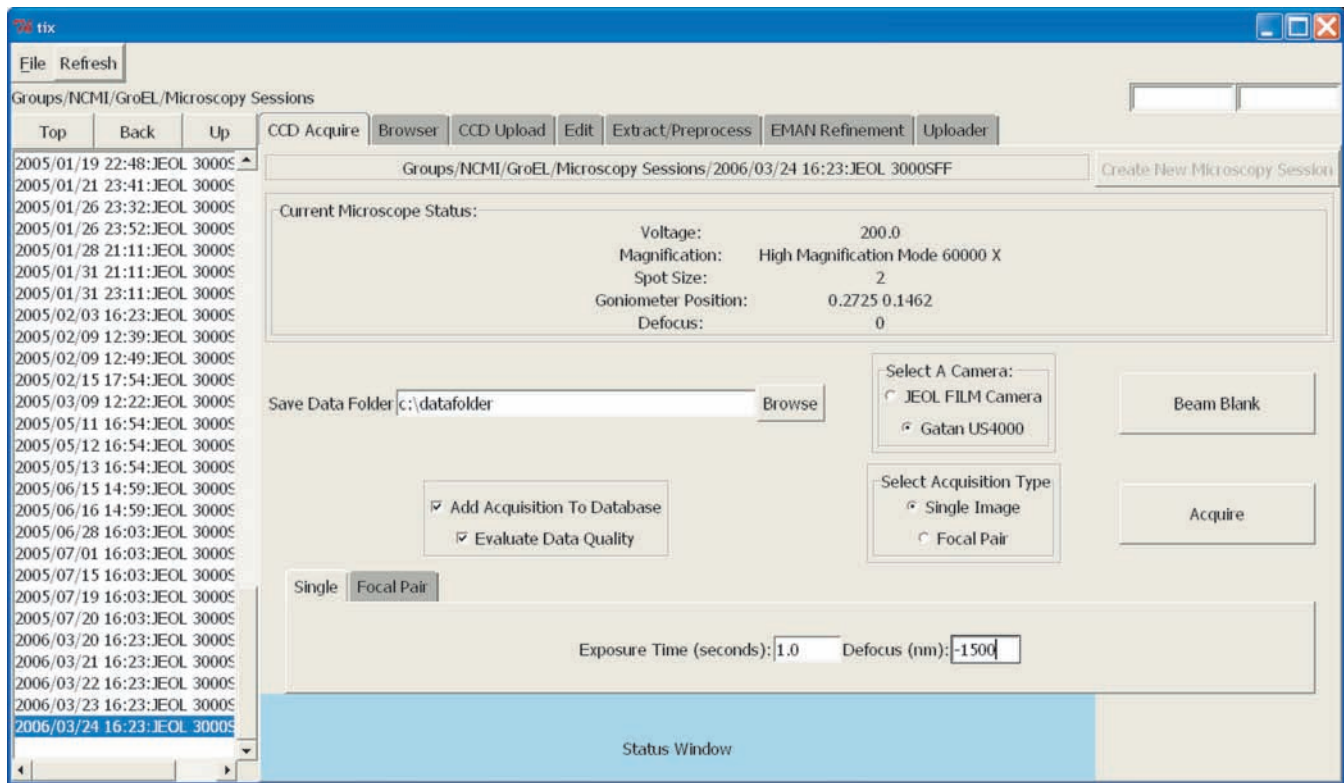


Fig. 2. GUI for JAMES single-particle application. The GUI is integrated with a view of the database. The panel on the left shows the available microscopy sessions in the database to which acquired micrographs should be attached. The main panel shows the status of the microscope and parameters that control the behaviour of the 'Acquire' button, such as choice of camera and choice of single exposure or focal pair. The tabs at the top of the interface correspond to unrelated operations that can be performed on database entries.

imaging by controlling the microscope and the CCD camera with attention to beam-blanking and incidental specimen exposure. Without software integration, the seemingly simple proposition of acquiring and saving a single image was surprisingly tedious. It required a ten-step procedure of navigating the interfaces of FASTEM, DM and the microscope console.

1. Console: select an area to image
2. FASTEM: engage the beam blank
3. Console: set the desired objective defocus
4. Console: raise the viewing screen
5. FASTEM: disengage the beam blank
6. DM: acquire the image
7. DM: enter a filename and save the image
8. DM: close the image
9. FASTEM: engage the beam blank
10. Console: lower the viewing screen

This procedure becomes more complicated if the user wants to take multiple exposures of the same area such as a focal pair. Because JAMES manages all of the devices for the user, the procedure is simplified. The JAMES interface allows the user to make a persistent setting for either one exposure or two, each at a prescribed defocus. Each time the user pushes the 'Acquire'

button on the JAMES interface, all of steps 2–10 described above are performed automatically, significantly reducing the repetitive tasks as follows.

1. Console: select an area
2. JAMES: acquire an image or focal pair

After the image is collected, the user can select the next area for imaging and repeat.

The example above omitted the step of recording the details of the acquired images (such as defocus, exposure time, grid position, etc.), which can obviously increase the time required for manual data collection. JAMES is integrated with the electron microscope electronic notebook, a database for archival of images and electron microscope metadata (Ludtke *et al.*, 2003). This integration permits JAMES to query the microscope for relevant metadata and then upload this data into the electron microscope electronic notebook in an automated way when acquiring images. The result is a more detailed account of the acquired images than a user is likely to record by hand.

Several projects have now been completed using data collected with the JAMES single-particle application (Booth *et al.*, 2004; Chang *et al.*, 2006; Jiang *et al.*, 2006a,b; Lee *et al.*, 2003; Ludtke *et al.*, 2004, 2005; Mao *et al.*, 2004;

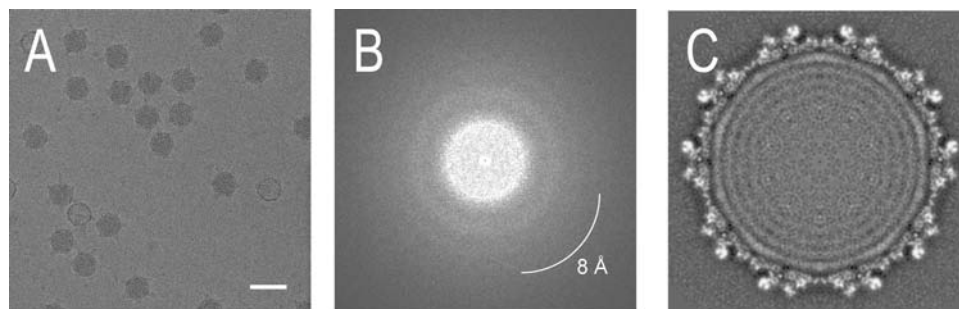


Fig. 3. Cytoplasmic polyhedrosis virus images collected by JAMES and corresponding reconstruction. (A) A 200-keV image of cytoplasmic polyhedrosis virus is acquired with this software. Following microscope alignment and setup of FASTEM minimum dose system, areas of interest were identified in search mode, and JAMES was used to record all high-resolution images in photo mode. Images were acquired as focal pairs. (B) Power spectrum of the first image as computed according to Saad *et al.* (2001). The signal-to-noise ratio extends beyond 9 Å. (C) A slice through the 9 Å resolution reconstruction. Scale bar represents 1000 Å.

Marsh *et al.*, 2006; Pope *et al.*, 2007; Yu *et al.*, 2005). Each of these projects consists of many single-particle images collected and used for reconstruction. In many cases the data collected with JAMES are sufficient for structural determination to subnanometer resolution. Figure 3(A) shows an example of using JAMES to record images of ice-embedded virus particles at $83\,100\times$ effective magnification onto the Gatan US4000 CCD camera and its corresponding power spectrum of the boxed-out particle images (Fig. 3B). A contrast transfer function ring is generally detectable beyond 9 Å after the background is properly subtracted from the 1D averaged power spectrum. In this project, JAMES software was used to acquire 428 focal pairs spanning five imaging sessions, resulting in 6465 unique particles that were used to compute an icosahedral reconstruction at 9 Å resolution (Fig. 3C), in which long alpha helices and large beta sheets can be identified (Booth *et al.*, 2004). The other projects followed similar imaging parameters.

MR T: a tomography application

Tomographic imaging is a process by which images of a single area are acquired while tilting the goniometer through a range of angles. Many groups have automated tomographic data collection (Daberkow *et al.*, 1996; Mastronarde, 2005; Nickell *et al.*, 2005; Zheng *et al.*, 2007; Ziese *et al.*, 2002). However, there is currently no computer software available for electron cryotomography of dose-sensitive specimens on JEOL microscopes running FASTEM software. Because the JAMES API INTEGRATOR offers a unified interface that can control stage tilt as well as image acquisition, we have used it to develop a software package called MR T to facilitate the acquisition of low-dose tilt series. The result, which includes a simple graphical user interface (GUI) (Fig. 4), permits routine acquisition of tilt series on a JEOL 2010F microscope, even by inexperienced users.

MR T offers many features offered in tomography solutions implemented by other investigators for their own instruments. To keep the area of interest in the field of view, MR T offers specimen tracking by cross-correlation or by precalibration (Ziese *et al.*, 2002). MR T's data collection routine permits flexibility in the angular sampling, allowing for fixed interval or Saxton interval tilting (Saxton *et al.*, 1984). Users can also choose to have all exposures acquired with equal dose or have their dose weighted by the secant of the tilt angle to compensate for the weaker signal-to-noise ratio at higher tilt angles, as implemented in SERIALEM (Mastronarde, 2005) and also described in methods of Medalia *et al.* (2002). MR T's approach to dose management allows the user to specify a total allowable dose, and then the software plans the dose partitioning for each exposure to meet these requirements without exceeding this limit.

Several biological samples have been imaged and reconstructed using MR T, including lipid vesicles (Chang *et al.*, 2005), cells (Marsh *et al.*, 2005) and Herpes Simplex Virus capsid (Chang *et al.*, 2007). Figure 5 shows our results from a 2-h session using MR T in manual-tracking mode to record a cryotomographic series of lipid vesicles at $34\,600\times$ effective magnification and total cumulative dose of $30\text{ e}^- \text{Å}^{-2}$. The IMOD reconstruction package (Kremer *et al.*, 1996) was used to generate a tomogram from 61 images spanning -60° to $+60^\circ$ in 2° increments. A single image from the tilt series (Fig. 5A) does not resolve the features of the vesicle organization. The tomogram reveals three nearly concentric vesicles that are annotated as cut-away spheres (Fig. 5C). A slice of the tomogram (Fig. 5B) shows the organization of these three vesicles, in which the leaflets of the bilayer are resolved.

Conclusion

Experimental science continues to push beyond the limitations of the instruments and the software. The JAMES platform was born out of the need to perform specific tasks with the

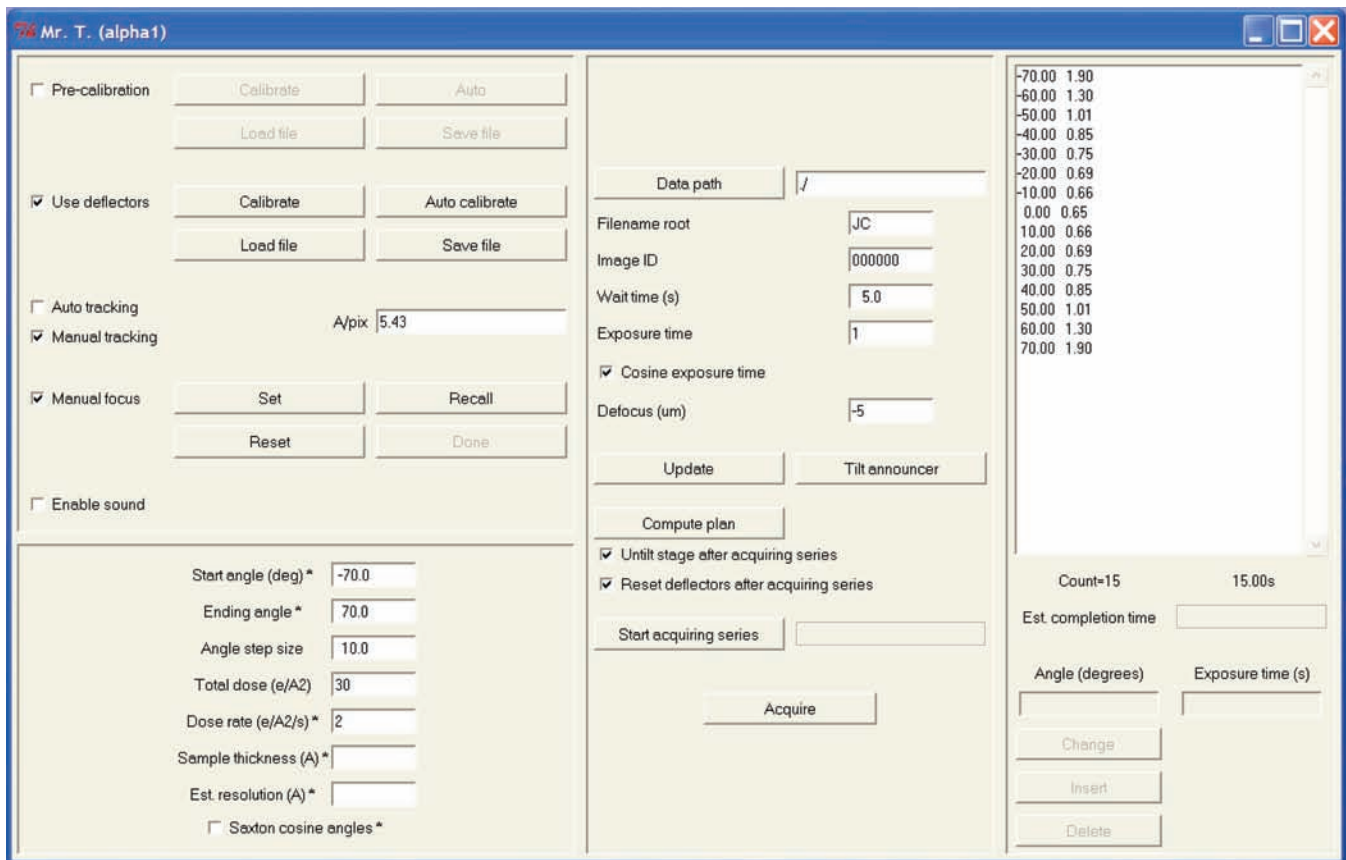


Fig. 4. GUI for MR T tomographic imaging application. The GUI allows the user to enter parameters for the tilt experiment, including the tracking method, tilt range, tilt step and exposure time. In addition, the tilt plan (rightmost panel) can be arbitrarily modified.

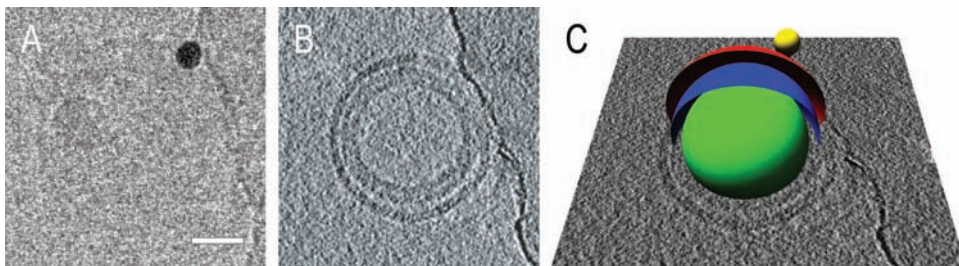


Fig. 5. Electron cryotomography of lipid vesicles. (A) Single image of frozen-hydrated vesicles and colloidal gold acquired with MR T at 0° tilt, 200 keV. A reconstruction was performed with the IMOD package using the colloidal gold for fiducial alignment. (B) A slice through the tomogram, reconstructed from 61 images, shows three concentric vesicles. (C) The same slice with hollow spheres modelling the position of the vesicles. The yellow sphere shows the position of the colloidal gold. Scale bar represents 300 Å.

transmission electron microscopy, and cryoTEM in particular, that were not well supported by commercial or publicly accessible software. By implementing a modular design for a core programming interface to control the microscope, we have achieved a multipurpose library with the JAMES platform. The library and the applications can be downloaded from our website (<http://ncmi.bcm.tmc.edu/software/james>).

We have shown how the library has been used to develop prototype applications for single-particle and tomographic

imaging of frozen-hydrated specimens of biological origin. These applications can be extended into feature-complete solutions, similar to automated applications reported for other instruments (Mastrorarde, 2005; Nickell *et al.*, 2005; Stagg *et al.*, 2006; Zheng *et al.*, 2007). The strength of the JAMES platform is its modularity, which permits replacement of any microscope component without changing the application layer but instead by merely implementing the low-level code for the new component.

Acknowledgements

The research has been supported by National Institutes of Health National Center for Research Resources (P41RR02250), National Institutes of Health through the NIH Roadmap for Medical Research (PN2EY016525), National Institute of General Medical Sciences (P01GM064676) and the Robert Welch Foundation. We thank Jaap Brink and Bob O'Donnell (JEOL), Jacob Wilbrink, Robin Harmon and Doug Hauge (Gatan) for their technical assistance. The vesicles were kindly provided by Ka Yee C. Lee and Guohui Wu (U Chicago).

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