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JADAS: A Customizable Automated Data Acquisition System and its Application to Ice-embedded Single Particles

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Abstract

The JEOL Automated Data Acquisition System (JADAS) is a software system built for the latest generation of the JEOL Transmission Electron Microscopes. It is designed to partially or fully automate image acquisition for ice-embedded single particles under low dose conditions. Its built-in flexibility permits users to customize the order of various imaging operations. In this paper, we describe how JADAS is used to accurately locate and image suitable specimen areas on a grid of ice-embedded particles. We also demonstrate the utility of JADAS by imaging the epsilon 15 bacteriophage with the JEM3200FSC electron cryo-microscope, showing that sufficient images can be collected in a single 8-hour session to yield a subnanometer resolution structure which agrees with the previously determined structure.

Keywords

Single-particle; Automated data collection; Reconstruction; Cryo-EM

Introduction

Advances in image reconstruction software have enabled researchers to solve three-dimensional structures of macromolecular complexes using single particle electron cryo-microscopy (cryo-EM) beyond 5 Å resolution (Jiang et al., 2008; Ludtke et al., 2008; Yu et al., 2008; Zhang et al., 2008). If the particles exist only in one conformation, it requires only few tens of particle images to reach a subnanometer resolution reconstruction (Borgnia et al., 2004; Liu et al., 2007). However, most of the biologically active particles -- in living cells or

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in vitro -- are flexible and heterogeneous. In order to separate those particle images into different classes of homogeneous conformations prior to the image reconstruction, large numbers of particle images are necessary (Chen et al., 2006; Scheres et al., 2007). Recent adoption of statistical methods to sort out the heterogeneous particles (Elad et al., 2008; Penczek et al., 2006a; Penczek et al., 2006b) requires large quantities of data to yield a meaningful statistical evaluation. Therefore, large datasets have become the norm, regardless of the targeted resolution, for many single particle studies.

The demand for such large datasets necessitates reliable and efficient automation of image data collection for single particle cryo-EM. Researchers in both industry and academic institutions have developed several software systems with various degrees of automation and robustness. *AutoEM* (Lei and Frank, 2005; Zhang et al., 2003) provides semi-automated capabilities for the FEI Tecnai series electron microscopes. It operates mainly at a low level of automation that requires substantial operator involvement (i.e. in selecting holes for imaging). *Leginon* is the most advanced automation package designed to provide fully automated functions for the Phillips CM series as well as the FEI Tecnai series of transmission electron microscopes (Carragher et al., 2000; Suloway et al., 2005). *SerialEM* (Mastronarde, 2005) is an automated electron tomography package for most contemporary FEI and JEOL instruments and, in principle, can be adopted for single particle imaging. *TOM Toolbox* includes tools for both data acquisition and processing for single particles and cryo-tomography in FEI microscopes (Nickell et al., 2005). FEI also offers commercial software - *Batch Tomography* (FEI Inc, USA) which was designed for cryo-tomography data acquisition.

For JEOL microscope, a trial to port *Leginon* to JEM3100FSC was reported (Glaeser et al., 2006) but no structural result is yet demonstrated. A semi-automatic software (*James*) was also developed to interface between Gatan DigitalMicrograph and the FASTEM computer interface in the JEM2010F microscope (Marsh et al., 2007). This software currently has limited applicability because FASTEM is no longer available for the newer JEOL microscopes. Based on the conceptual approach of *James* designed for both experienced and novice users, we have developed JADAS (JEOL Automated Data Acquisition System) for present and future models of JEOL instruments in order to translate our high resolution imaging experience into an industrial grade software product.

In this paper we introduce the JADAS automation system, including a discussion of its design and validation of its usefulness. We also present an example of high-quality image data acquired using JADAS on an ice-embedded single particle specimen – epsilon 15 bacteriophage which has a known cryo-EM determined structure (Jiang et al., 2008).

Design of JADAS

1. Connectivity between JADAS, microscope, camera and accessories

JADAS is written using the C# programming language. It links specific software and drivers in order to concurrently control the operations of the microscope, camera and other accessories. As shown in figure 1, JADAS communicates with the JEOL microscope through the TemServer software, with a Gatan CCD through DigitalMicrograph, and with the piezo controlled stage (if present) through the specimen piezo driver. It is designed in such a way that its functionality can be extended to other hardware and associated drivers.

2. Low dose imaging

JADAS is designed mainly for imaging ice-embedded biological specimens using Quantifoil® (Quantifoil Micro Tools GmbH, Germany) or equivalent type of grids (e.g. C-flat™ Protochips, Inc., USA) and can be adapted to various imaging protocols. A typical cryo-EM low dose

protocol consists of 4 steps (Figure 2): 1. A global search for the grid squares with potentially suitable imaging areas; 2. A finer, local search for specimen areas suitable for imaging within the grid squares selected in step 1; 3. Setting up microscope parameters such as objective lens defocus and specimen drift compensation; 4. Recording images at a user specified dose, magnification and defocus.

Step 1 is an initial step while steps 2-4 run in an iterative cycle for each grid square identified in step 1. JADAS performs these steps using fully or partially automatic modes of operation. The steps in this low dose protocol are generic, although the specific algorithms to carry out these steps in JADAS differ from other automation software packages. The algorithms implemented in JADAS are described below.

3. Global and local search algorithm

JADAS uses a multi-scale specimen searching algorithm to find potentially suitable areas for imaging within a grid. It first performs a coarse search of grid squares (global searching) followed by a fine search of suitable holes in the selected grid squares (local searching). In both steps, there are several choices of different degrees of automation that can be selected by the user.

In the montage-assisted global search mode, we assume a repeating square mesh grid type. A low magnification montage (e.g. 100X or 150X magnification) of contiguous areas on the whole grid is produced (Figure 3). Using this montage image, the locations of each grid square are determined based on its periodicity. Since simple cross-correlation or thresholding tends to fail to detect precisely each grid square, a Fourier transform is calculated from the montage image and the direction and spacing of the square array are estimated from the fast Fourier transform amplitude peak position. After this, a reference small array of square image is generated with the same direction and spacing, and actual position of each square in the montage image is determined by cross-correlating with this reference. This montage-assisted global search mode provides the user with a broader view of the extent of the usability of the entire grid. In this mode, JADAS automatically detects and identifies candidate grid squares for calibration or data collection. The image intensity histogram of all the identified grid squares is displayed (Figure 3 upper right panel). The user then sets the image intensity thresholds corresponding to a suitable ice thickness according to the thickness of the specific specimen. These thresholds instruct JADAS to skip squares with average intensity levels that are too low or too high. JADAS also provides a manual mode which allows the user to select grid squares interactively, which can be useful if only a small number of suitable imaging regions exist. JADAS maintains a list of the targeted grid squares for later use

Alternatively, the user can manually move the grid systematically at a higher magnification using the selected area diffraction mode or a normal mag mode (e.g. 2,000X) to hunt for grid squares that appear suitable for data acquisition.

After completing the global search of suitable grid squares, JADAS moves the microscope stage to each selected grid square and carries out the sequence of local search and imaging (i.e. step 2-4). The user can direct JADAS to automatically select areas for microscope parameter fine-tuning (focusing, drift compensation, and astigmatism correction) and for imaging on holes with an acceptable image intensity threshold. Alternatively, JADAS permits manual selection in local areas for fine-tuning the microscope settings and for acquiring images respectively.

In the automatic local search mode, the microscope can be set to either selected area diffraction mode at a camera length (e.g. 150 cm) with the electron diffraction pattern highly defocused (refer hereon to blurred diffraction mode) or normal mag mode (e.g. 2,000X). The benefit of

using blurred diffraction mode for search mode is that a relatively wide view range is available while the objective lens is kept activated to minimize its hysteresis when switching between search and focus steps. However, using the blurred diffraction mode creates a large distortion in the search step image which makes the automated step of finding the hole positions difficult. JADAS overcomes this problem by correcting the geometrical distortion. The parameters to correct the image distortion in the blurred diffraction mode include the direction of the hole array, the diameter of a hole, the distance between neighboring holes, the center and the extent of the distortion, and the image intensity threshold for a good ice hole. They will be calibrated at the beginning of each microscope session using a graphical interface provided by JADAS. The location of each hole is determined by a template-based search algorithm to recognize positions on repeating arrays of holes (N. Nakamura, manuscript in preparation). After manually defining the image intensity criterion, the holes can be automatically selected without human intervention (Figure 4a). Once there are no more holes which satisfy the intensity criterion in the current search step view, JADAS moves the stage to the next chosen grid square and repeats the local search again until it travels through all the grid squares selected in the global search step or until the user suspends the operation.

It is also possible for the microscope operator to manually select the local grid holes for imaging (a semi-automatic approach). This is done through an interactive positioning tool. When using the interactive positioning tool, JADAS first acquires several search step images. The user can then manually select areas for fine-tuning the microscope and for recording images (Figure 4b). Usually the user selects all the positions at the beginning of the imaging session. JADAS stores the selected positions in memory and then moves the microscope stage to each selected position to carry out the operations (fine-tuning, imaging, etc). This manual procedure is particularly useful for grids with irregular holes.

4. Auto fine tuning

JADAS uses two common methods for determining defocus: a beam-tilt (Koster et al., 1987) or a diffractogram based method (Saxton et al., 1983). To compensate for stage drift, the user can either wait until the stage is stable or use the piezo stage for drift compensation (Kondo et al., 1994).

JADAS can analyze a diffractogram for stigmatism correction also. When the specimen is on a holey carbon film, the software can automatically adjust the objective lens stigmator by measuring the ellipticity of the contrast transfer function rings to correct the astigmatism (Saxton et al., 1983).

5. Recipe and recipe editor

To maximize the flexibility for different imaging applications, JADAS adopts the concept of reusable “recipes,” which can be customized according to the needs and purpose of an experiment. Each recipe contains one or more *steps* where each *step* can have multiple *operations* (Figure 5). The repetitive low-dose imaging procedure (Figure 2) can be described in terms of a recipe containing 3 *steps* (Figure 6 right panel). The *operations* (Figure 6 lower left panel) are pre-defined in JADAS but can be expanded through the JADAS API. Examples of *operations* are Capture (expose the sample to electron beam and record the image), Defocus (include Set Defocus, Randomize Defocus, Focal Pair, etc), Blanking / un-blanking the beam, Open / close the fluorescent screen, Fine-tuning the microscope, Area searching strategy (such as manual search, interactive positioning and intensity based hole selection), etc. All the *operations* within a single *step* share the same *condition parameters* which define specific lens, deflectors and camera settings for either the CCD or the photographic film recording.

JADAS provides a graphic user interface (Recipe Editor, see Figure 6) to create, edit or save a recipe in an xml file format. In the recipe editor, one can predefine all the **condition parameter** settings for the microscope lens, deflectors (Supplement Figure 1) and the camera (Supplement Figure 2a,b). One can also leave some of the values undefined and set them up at a later stage during setup and calibration. Those **operations** will be carried out under the **condition parameters** which have been defined.

To create a recipe, the user can select from pre-defined templates for **Condition Parameter Settings** (Figure 6 upper left panel of recipe editor) and **Operations** (Figure 6 lower left panel of recipe editor).

The receipt editor is designed for experienced users who want to have more control of the data collection or develop new imaging protocol for their particular needs. For novice users, they can use immediately one of the established recipes for routine single particle data collection at different automation levels.

Experimental Methods

The microscopes used to test and validate JADAS were JEM3200FSC (300kV) and JEM2100 (200kV). Both of them are equipped with a Gatan 4k×4k CCD camera (Booth et al., 2004; Chen et al., 2008). The JEM3200FSC has a field emission gun while the JEM2100 has a LaB₆ gun. The grids used in all the experiments are Quantifoil® 1.2/1.3 400 mesh grid. The frozen, hydrated single particle specimens were prepared using the Vitrobot (FEI Inc, USA). The biological specimens used for the testing include epsilon15 bacteriophage (Jiang et al., 2008) and Methanococcus maripaludis chaperonin (Mm-cpn) (Reissmann et al., 2007). The effective magnifications of the experiments varied between 28,200X and 56,680X.

Results and Discussion

1. Installation of JADAS

JADAS is developed explicitly for the current generation of JEOL instrument such as JEM3200, 3100, 2100 and 2200. All of the tests reported here were done in the JEM3200FSC and 2100 microscopes and no special modification of the software has been encountered to run on either instrument. JADAS runs only on Windows XP operation system with at least 1GB memory and it must be connected to a new type of JEM series that has the TemServer software. The specimen must be frozen on arrayed holes when the user wants to collect data fully automatically.

Current version of JADAS runs only with Gatan camera system or the JEOL photographic film camera system. However, it can be ported to other cameras with a modification of a camera control software unit. Piezo control unit is an optional hardware to compensate for the stage drift actively, but it is not required to run JADAS. No modification of the existing microscope is needed to operate JADAS.

2. Criteria for specimen area selection

The choice of grid square and holes to be used for imaging is based on the image intensity estimated by the user to be an appropriate ice thickness. The image intensity threshold is specimen and voltage dependent. In addition, the apparent image contrast can be affected by the electron optical set up at the initial search mode. Therefore, JADAS allows the user to define the image intensity threshold for choosing candidate holes for a specific experiment. In the case of an electron microscope with an energy filter such as JEM3200FSC, it is possible to measure the ice thickness quantitatively by using a pre-calibrated value of mean free path of the ice for a specific electron optical condition, and such implement does not yet exist.

JADAS allows the user to pick holes for imaging manually or automatically. The automated hole detection can work only with periodically arrayed holes like Quantifoil® or equivalent types of grids. The user is prompted to carry out a calibration before data collection using a graphical interface. The calibrations are used by a template based hole matching algorithm to detect each hole in a grid square (N. Nakamura, in preparation).

3. Accuracy of returning to the previously selected holes

The accuracy of returning to the previously selected holes can depend on the type of cryo-holder as well the microscope. To validate the stage positioning accuracy of JADAS we performed a test using the following recipe: JADAS automatically identified holes in a grid square according to the image intensity criterion; traveled to each of the chosen holes and took images with the centers of holes positioned at the center of the CCD frame at 28,200X effective magnification. Under this magnification, each hole was expected to fall within the CCD frame. To measure the precision of the positioning of the recorded holes, we measured the deviation of the center of each hole from the center of the CCD frame (Figure 7a).

The tests were carried out on the JEM2100 electron cryomicroscope with a LaB₆ gun and a 70 degree Gatan cryo-specimen holder (model 626). In this experiment, 103 image frames of Mm-cpn particles were recorded. Analysis of the frames revealed that 93% of the hole centers fell within the distance less than 0.3 μm from the CCD frame centers (Figure 7b). These deviations might be caused by backlash of the stage or imperfections in the distortion modeling for the blurred diffraction image used in the search mode. Similar performance is found also in the JEM3200FSC with the JEOL cryo-holder (see results in section 4).

At a magnification suitable for $\sim 6\text{\AA}$ resolution reconstruction ($\sim 83,400\text{X}$ effective magnification or approximately $1.83\text{\AA}/\text{pix}$ (Booth et al., 2004)) a $4\text{k}\times\text{k}$ CCD will cover about $0.7\text{\AA} \times 0.7\text{\AA}$ area on the specimen grid. On a Quantifoil® 1.2/1.3 grid (with a hole diameter of $1.2\text{\AA}$), a distance of $0.3\text{\AA}$ between the hole center and the CCD frame center will still guarantee most of the CCD image taken within the hole.

4. Biological application

A biological application of JADAS using the epsilon 15 bacteriophage was carried out using a JEM3200FSC at 56,680 X effective magnification. This magnification was used because we aimed at subnanometer resolution data using the Gatan $4\text{k}\times\text{k}$ CCD camera, with structure can be reconstructed to 2/3 Nyquist frequency of the camera space or $\sim 8\text{\AA}$ in the object space (Chen et al., 2008). The energy filter was set open for the search step and automatically inserted for the focus and photo steps with the slit width of 10eV. All the holes used for focusing and imaging were automatically selected using the automatic local search. A 45-second delay was set before taking each CCD frame to maximize the stage stability after the stage is moved to a new specimen area.

One hundred eighty-one CCD frames of epsilon 15 bacteriophage were taken by JADAS over an 8-hour session on the JEM3200 microscope (including about 30 minutes for initial microscope alignment, JADAS setup and hole selection, and about 30 minutes for refilling the liquid nitrogen once during the microscopy session). Figure 8 shows an example image acquired by JADAS showing epsilon 15 bacteriophage particles embedded in vitreous ice.

The particles were automatically boxed out using ethan (Kivioja et al., 2000). The parameters for the contrast transfer function was first automatically fit using *fitctf.py* in EMAN (Ludtke et al., 1999; Ludtke et al., 2001) and then manually examined using CTFIT.

When applied to these CCD image frames, `fitctf.py` acceptably fit the parameters of the contrast transfer function for 155 of them (~86%). The remaining 26 CCD image frames had either too few virus particles per frame or too low contrast due to small defocus values.

Those 155 CCD frames, which were further processed, contained 7,543 particle images. Image reconstruction was performed using the multi-path simulated annealing algorithm (Liu et al., 2007). The structure was resolved to 7.3 Å resolution (Figure 9a; Supplement Figure 3), according to the 0.5 FSC criteria, by comparing to our epsilon 15 structure previously determined from data acquired manually on photographic film (Jiang et al., 2008). One monomer of the protein gp7 was segmented out. 7 of 8 expected α -helices and 3 of 3 β -sheets were detected (Figure 9b,c) using SSEHunter (Baker et al., 2007). Such highly accurate structure determination validates the usefulness of JADAS for actual data collection of ice embedded single particles.

5. Recipe strategy

When designing JADAS, we envisioned its practical usage to be flexible depending on the grid and preferences of the user. Some users may prefer to have more control on the choice of the specimen area and use JADAS to do the focusing (as shown in Figure 4b), while others may prefer to let JADAS perform the entire step from hole searching to image acquisition. Such completely automated procedure has already been successfully applied to other ongoing projects.

Within a JADAS recipe, there can be variations on the protocol when choosing the method of focusing. In a situation in which the grid is like the above examples, it is not necessary to focus before imaging each hole; performing the focus operation in a few areas within a grid square is sufficient. JADAS can use different defocus values (either through “Randomize Defocus” operation which JADAS can randomly choose a defocus value within the defocus range set by user or by specifying different defocus values) for the neighboring holes relative to the hole used for focus setup. This procedure will speed up the data collection process. However, if the grid is bent, large defocus variations will occur in the adjacent areas and it will necessitate frequent focusing even within a single grid square; this will increase the length of time needed to acquire a large data set.

Other possible recipe applications of JADAS include recording electron diffraction patterns followed by images of protein crystals or recording a series of images or electron diffraction patterns from a single specimen area in a typical radiation damage experiment.

6. Modularity and flexibility of JADAS

JADAS is developed for the current generation of JEOL instruments. It is impractical to adapt it to the older generation of JEOL instrument such as JEL2010 and 3000 series because of the significant differences in the computer interface to the microscope controls. Though JADAS is now programmed to run on either the JEOL film camera or the Gatan CCD camera, it can be easily modified to connect to camera systems of other sorts. The current JADAS allows the users to collect images of ice embedded single particles using existing and well-tested recipe protocols that come with JADAS. Alternatively, the users can modify the recipe to conduct different types of experiments. For some instruments, cryo-specimen drift can present a serious technical hurdle to collecting high resolution images. In fact, JADAS offers an option of active drift compensation with piezo if it is available in the instrument.

There can also be numerous extensions of the current operation menu of JADAS. For instance, one may incorporate a quantitative measurement of the ice thickness using the energy filter available in some models of JEOL electron cryomicroscopes. The capability of JADAS can

be enhanced further by coupling it with other tools developed elsewhere. For instance, by integrating with the newly developed database EMEN2 (Tu et al., 2006), JADAS-acquired data, along with the imaging condition parameters, can readily be uploaded to the database while data collection is still in progress. Another possibility is to integrate image processing function from EMAN to assess the data quality in real time, thereby giving researchers immediate feedback during data acquisition. By combining the utility of off-the-shelf remote logon software (e.g. WebEX: <http://www.webex.com/>), researchers can also monitor the data collection process and even operate JADAS remotely. Therefore, JADAS represents the type of data collection software which will satisfy multiple modes of applications.

7. Practical considerations in automated data collection

A primary purpose in automated data collection is to acquire a large amount of data with uniform data quality at a fastest speed. JADAS and other similar software are achieving this goal if the grid is good in terms of particle distribution and uniform ice thickness, and the instrument is in optimal performing condition. Unfortunately, these conditions may not always be met in a day-to-day operation with many different types of specimens in a typical EM lab. For instance, a frequently encountered problem is the uneven particle distribution in the holes across different parts of the grid though the ice thickness appears fine. At the moment, an experienced user is the best and the quickest to make the judgment whether it is worthwhile to continue the experiment, choose the right area on the grid to image or change another grid. In addition, there are always “on-site” decision regarding the acceptable level of ice contamination for a particular grid and the stability of the cryo-holder. Therefore, the presence of trained user either on-site or off-site through remote microscope access is still necessary to assure the data quality before the experiment to continue with a given grid and not to waste time in collecting useless data.

For a large data set, the speed of data collection is a concern. Though recording a CCD frame is a matter of ~5 seconds (on a 4k×4k CCD) including data capture and archiving on the disk. The most time limiting steps are the quantitative assessment of the suitable specimen area and the defocus setting for each frame in addition to the wait time needed between images of different specimen area to assure stage stability. In our approach, we adopt the strategy not to measure the defocus for each recorded frame. This will work if the grid is flat and sits evenly in the microscope column. In our experience with JADAS operated with the JEM3200FSC, we can record 30-40 4k×4k CCD frames per hour in the condition with both excellent grid quality and optimal instrument performance. The quality of images is uniformly good as shown in Figure 8-9.

The estimate of the achievable resolution in the images acquired by the Gatan 4k×4k CCD camera with the 300 kV electron is $\sim 2/3$ Nyquist frequency of the camera (Chen et al., 2008). In the biological example shown here, the resolution of the reconstruction is $\sim 7.3\text{\AA}$ which exceeds the anticipated limit. In order to extend the resolution further, one needs to increase the magnification more. The higher the magnification is used, the less number of particles per CCD frame would be recorded. For instance, a 4-5 $\text{\AA}$ reconstruction map of a molecular machine of $\sim 1\text{ MDa}$ with a single rotational symmetry may need a pool of $\sim 100,000$ particle images. If each CCD frame records ~ 100 particle images, this will require $\sim 1,000$ CCD frames. At the speed of ~ 40 frames an hour, this will take ~ 25 hours of data collection. By taking into account of the set-up and liquid nitrogen refill, this can be done in 2 days. Such rate is longer than a complete data collection for an x-ray crystal in a synchrotron beam line. But, cryo-EM does not require heavy atom or other additional measurements. Therefore, the cryo-EM data collection using automation method as illustrated here may no longer be an insurmountable task to collect data for a backbone trace of a molecular machine after the grid and sample preparation is fully optimized for a given specimen. Therefore, the current time limiting steps

in cryo-EM structure determination lie at the steps of biochemical specimen preparation, cryo-specimen preservation, data processing and structure interpretation.

Conclusion

A flexible computer controlled data acquisition system is highly desirable in biological cryo-EM because the experimental protocol may vary depending on the purpose of the experiment and user preference. The JADAS recipe concept offers experienced users a high level of flexibility to build their own experimental protocols while it gives novice users standard procedures to follow with step-by-step aids. The current study with the assessment of the positional accuracy of the targeted holes for data recording (Figure 7) and the actual data collection of epsilon 15 bacteriophage (Figure 8) demonstrate the utility of the JADAS recipe.

In testing the robustness of JADAS during development, we used epsilon 15 bacteriophage with a known structure (Jiang et al., 2008). We used the automatic mode to select holes and record a set of images in JEM3200FSC cryomicroscope within one day to yield sufficient images (Figure 8) to reconstruct an accurate subnanometer resolution structure at which secondary structure elements can be identified (Figure 9). The automatic hole selection with the ice-embedded Mm-cpn particles was also evaluated systematically in the JEM2100 microscope equipped with a standard Gatan cryo-holder as shown in figure 7.

We have described the first example of software for fully automated cryo-EM data collection on modern JEOL microscopes. JADAS is a highly customizable system and it is likely that the recipes of JADAS will continue to expand to support future hardware and software improvements. The flexibility of the JADAS design also can allow users to either adopt the available recipes or to create new recipes customized for their own experiments, without the direct help of a software developer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Connectivity between JADAS and the drivers of different microscope hardware

Figure 2.

Typical cryo-EM low dose operation and the composition of a JADAS recipe. The cryo-EM low dose operation starts from a global search of the grid squares for potentially suitable area; then moves to the chosen grid square to perform a local search for potentially usable holes; then moves to the chosen hole to set up microscope parameters and take an image (or images) before moves to another chosen hole. After traveling through all the selected holes in the grid square, JADAS will move to another selected square and repeat the steps in the meshed box. JADAS allows extensive customization of those steps within a “recipe”.

Figure 3. Grid Square Selection GUI for montage-assisted global search. The montage image at low magnification is displayed on the upper left. Detected grid squares are marked in colors on the montage image. The brightness histogram of the squares is shown on the upper right and the user can move sliders to set the threshold for squares selection (light blue) or discard (light peach). User can also manually select squares (green) for data acquisition, mark squares (red) to not to take images and choose a square (bright yellow) for calibration.

Figure 4.

a. Intensity Based Hole Selection GUI for automatic local search. Green circles mark those holes that meet the preset image intensity criteria. Light peach circles mark those holes that do not meet the criteria. Magenta cross indicates the hole that is being imaged at the moment. **b.** Manual Interactive Positioning GUI. User can manually select the positions (green) for focusing or the positions (magenta) for imaging.

Figure 5.

Concept of a recipe. A recipe is composed of multiple steps. Each step contains a sequence of different *operations*. These *operations* within one step are carried out under the condition defined by the Condition Parameter Set (such as the lens and deflector setting, the CCD setting, etc.).

Figure 6.

Recipe Editor and a sample recipe. The User can select from JADAS provided *conditions* and *operations* (left panel) to design a recipe (right panels). The example recipe in the right panel consists of search, focus and photo steps. Each step contains a set of *conditions* and a set of *operations*. In this recipe, JADAS will detect holes of a predefined image intensity, automatically fine-tune the microscope, randomly set a defocus value within the defined range and capture a single frame on CCD.

Figure 7.

a. CCD frame of the ice-embedded Mm-cpn particles under 28,200X effective magnification in the JEM2100 microscope. Under this magnification, the entire grid hole could be covered by the CCD frame. The center of the hole and the center of the CCD image frame were marked (white dots). The area that can be covered by the 4k×4k CCD under the effective magnification of 56,400X, 84,600X and 112,800X in the JEM2100 microscope are marked respectively (black dashed squares). **b.** Distribution of distances between the centers of the 103 holes from the center of the CCD image frame.

Figure 8.

JADAS-acquired CCD image frame of epsilon15 phage particles embedded in vitreous ice on a Quantifoil® grid at 56,680X effective magnification in a JEM3200FSC cryomicroscope with an in-column energy filter.

Figure 9.

a. 3D map of epsilon15 bacteriophage at 7.3 Å resolution. **b-c.** One of the segmented gp7 proteins viewing from inside the virus capsid (b) and from outside the virus capsid (c). 7 α -helices (green) and 3 β -sheets (blue) were detected by SSE-Hunter.