Figure 5:*Creation of image stacks.* Users are able to create an image stack from the results of a particle selection routine, filtering the particles by the confidence of the CTF estimation, the particle correlation (if an automatic particle picker was used), or by defocus limits. Popup documentation is displayed describing the "box size" parameter. The resulting stack is summarized online, with an averaged image of all the particles comprising the stack. From this point the user may examine the individual particles within the stack, center the particles using EMAN'scenalignint, or create a subset of the stack.

Supplementary Figure 1:*From projects to experiments.* The project database (A) stores information about all projects at NRAMM, and can be viewed from the project web pages. Clicking on the name of any project will navigate to a page with information specific to the project, and provide a list of all Leginon data acquisition experiments that have been run (B). A brief experiment description is displayed along with the total number of images collected, and the duration of the experiment. Clicking on the experiment name will bring up the Leginon image viewer, by which users can examine all the images collected during the experiment (C). Clicking on the "summary" link will guide users to a page containing summarized plots and charts describing the data collected for that experiment (D).

Supplementary Figure 2:*Results of reference-free classification.* Initial alignment of the particles is performed through a web form, and the resulting eigenimages can be selected for classification through the "average particles into classes" link on this page. The classification results and variances can be viewed online by the appropriate links that appear upon completion of the processing.

Supplementary Figure 3:*Launching an Appion EMAN reconstruction.* Users create a refinement schema that combines procedures from EMAN with other processing steps. A. Clicking on this button will set defaults for the first iteration based on the number of particles in the selected stack and its box size and particle radius. B. The parameters for all completed reconstructions pertaining to the current project are displayed in this pull-down. Selection of any of these reconstruction schemas will populate the form with an identical set of iterations and parameters. C. This row displays the current parameters available for the selected reconstruction, with mouse-over popup documentation. D. The entered parameters for one iteration of refinement. E. Selecting this button will create a duplicate iteration that will appear below the selected iteration. F. The status of the reconstruction as it proceeds can be viewed online.

Supplementary Figure 4. The exemplar reconstruction summary. Upon completion of a 3-dimensional refinement, the user selects the most well-refined density from the reconstruction report page, which creates this summary page.

All information and metadata leading to this reconstruction are summarized, with a textual summary appearing at the bottom.

References

Bottcher, B., S.A. Wynne, and R.A. Crowther, 1997. Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. Nature 386: 88-91.

Cheng, A., D. Fellmann, J. Pulokas, C.S. Potter, and B. Carragher, 2006. Does contamination buildup limit throughput for automated cryoEM? J Struct Biol 154: 303-11.

Conway, J.F., N. Cheng, A. Zlotnick, P.T. Wingfield, S.J. Stahl, and A.C. Steven, 1997. Visualization of a 4-helix bundle in the hepatitis B virus capsid by cryo-electron microscopy. Nature 386: 91-4.

Fellmann, D., J. Pulokas, R.A. Milligan, B. Carragher, and C.S. Potter, 2002. A relational database for cryoEM: experience at one year and 50 000 images. J Struct Biol 137: 273-82.

Frank, J., M. Radermacher, P. Penczek, J. Zhu, Y. Li, M. Ladjadj, and A. Leith, 1996. SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields. J Struct Biol 116: 190-9.

Grigorieff, N., 2007. FREALIGN: high-resolution refinement of single particle structures. J Struct Biol 157: 117-25.

Harauz, G., and M. Van Heel, 1986. Exact filters for general geometry three dimensional reconstruction. Optik 73: 146-156.

Hohn, M., G. Tang, G. Goodyear, P.R. Baldwin, Z. Huang, P.A. Penczek, C. Yang, R.M. Glaeser, P.D. Adams, and S.J. Ludtke, 2007. SPARX, a new environment for Cryo-EM image processing. J Struct Biol 157: 47-55.

Jiang, W., M.L. Baker, J. Jakana, P.R. Weigele, J. King, and W. Chiu, 2008. Backbone structure of the infectious epsilon15 virus capsid revealed by electron cryomicroscopy. Nature 451: 1130-4.

Lander, G.C., A. Evilevitch, M. Jeembaeva, C.S. Potter, B. Carragher, and J.E. Johnson, 2008. Bacteriophage lambda stabilization by auxiliary protein gpD: timing, location, and mechanism of attachment determined by cryo-EM. Structure 16: 1399-406.

Ludtke, S.J., P.R. Baldwin, and W. Chiu, 1999. EMAN: semiautomated software for high-resolution single-particle reconstructions. J Struct Biol 128: 82-97.