

## HIGH THROUGHPUT IMAGE ACQUISITION USING THE LEGINON SYSTEM

J. Pulokas, C. Suloway, D. Fellmann, Y. Zhu, A. Cheng, J. Quispe, F. Guerra, S. Stagg, C.S. Potter and B. Carragher

National Resource for Automated Molecular Microscopy, Dept. of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037.

We have developed a system called Leginon to emulate all the decisions and actions of a highly trained microscopist [1]. The system is responsible for every aspect of image acquisition and can run unattended for over 24 hours. Leginon has been in regular use in our facility for several years. It has been used in the acquisition of over 500 data sets containing several terabytes of data. Recent work has concentrated on stabilizing the software, increasing throughput, and dissemination of the software to more facilities.

Two of the current challenges in cryo electron microscopy (cryoEM) are to improve the resolution of structure determination and to address the problem of conformational heterogeneity in a population of single particles. Both of these challenges are likely to require a large scale up in the numbers of particles that are acquired and analyzed. We have been using Leginon for data collection in cryoEM and integrating this system with the subsequent processing and analysis procedures required to reconstruct a 3D density map from the projection images obtained from the microscope. We have recently demonstrated that the system is capable of acquiring more than 100,000 particle images in a single 24 hour period. The tight coupling between the acquisition and analysis software and a relational database that manages the data allows a detailed analysis of the parameters and conditions associated with the images.

The Leginon system is now fairly mature and stable, and one of our goals is to transfer the technology to other groups enabling them to acquire data using their own instruments. The dissemination of the system is being accomplished via a series of week-long training workshops that are normally held at our own facility, although in several cases, a training team has also visited external facilities. During the internal workshops the participants are initially trained on the system using the infrastructure at our Resource. They then install the entire system on their own individual laptop computers and use this system to connect to our microscopes. By the final day each group has completed a full set of calibrations and acquired a dataset using a complex multiscale data acquisition application. The laptop system then provides a stable starting point at the home facility when connecting to a new instrument. Support is provided during this phase using phone and email contacts and requests for bug fixes and new features are managed using a web-based facility called Bugzilla. The system is fairly extensively documented, about 150 pages of documentation have been written so far and include installation instructions, training examples and detailed descriptions of the applications. So far 10 groups have been trained at internal workshops and 2 groups have been trained at remote facilities. The only minor problem encountered during training has been in installing the system across a very wide variety of laptop configurations and in getting the entire system (including the application, the database and the web based data server) running on a small laptop with limited memory capacity. The system is now running at 5 external locations on both Tecnai and Jeol microscopes.

The next phase of our work will be to continue optimizing the system for further increases in throughput. We will continue the dissemination of the system, provide support to the external

groups and to develop new applications for the system requested by the external users. .

## References

- [1] Carragher et al. (2000) JSB. 132, 33-45.
- [2] This work is supported by the National Institutes of Health through the National Center for Research Resources' P41 program (RR17573).

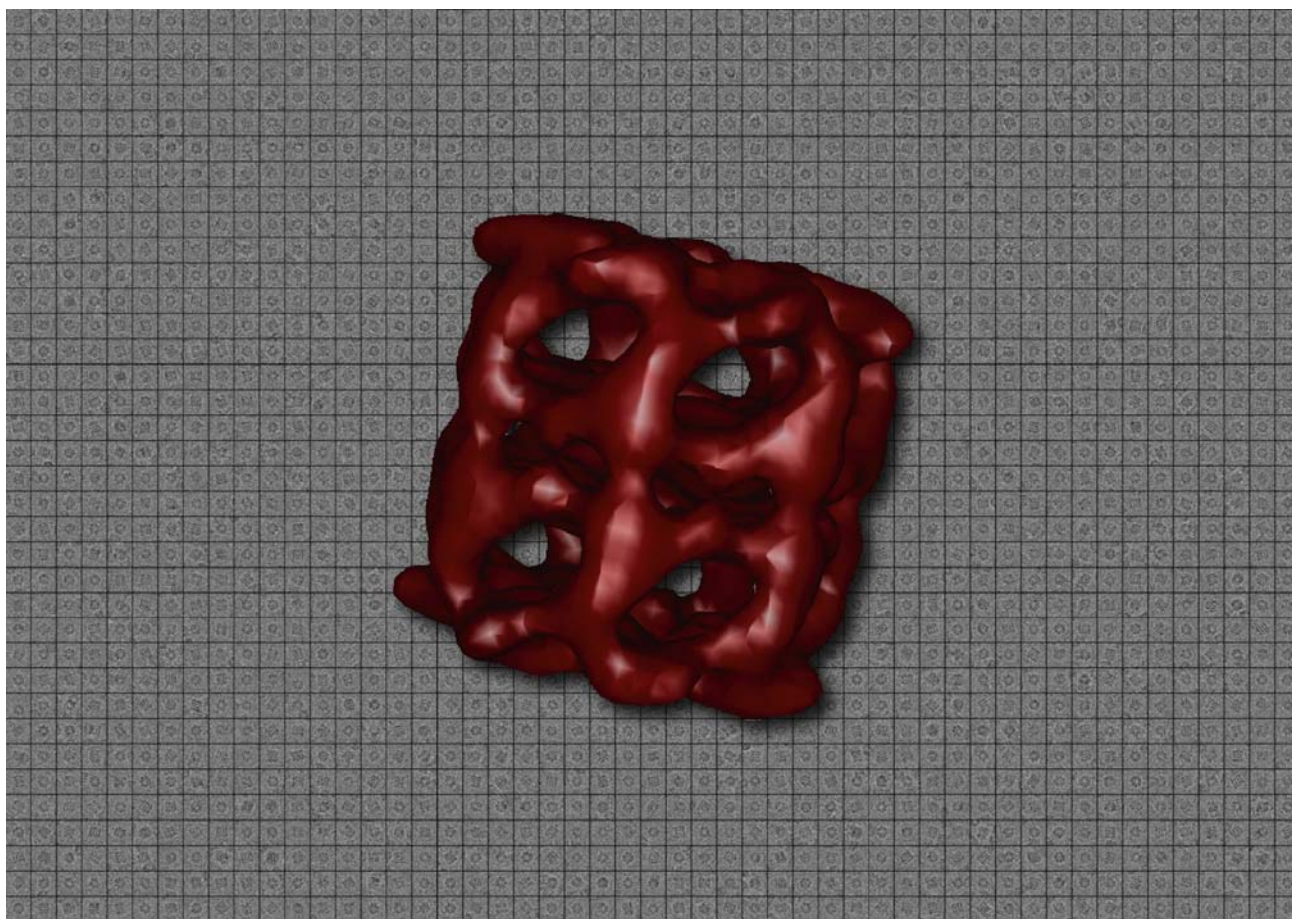


Fig. 1. Legimon was used to acquire over 100,000 GroEL particles in less than 24 hours. Shown are some of particles and a preliminary electron density map.