Visualizing Molecular Assemblies and Cells by Electron Microscopy

Tom Goddard
November 19, 2007

Topics

Progress this year

- Software for interactive exploration of EM tomography.
- New tools for single particle reconstruction -- our main base of EM users and collaborators.
- Advertising Chimera EM capabilities: meetings and journal articles.

Focus areas for next year

- Density map masks: file formats, creating and applying masks
- Tomography filtering operations: bilateral, median, anisotropic diffusion.
- Seamless user interface for atomic models of molecular assemblies.
Chimera Use in the Electron Microscopy Community

Who are our current EM users?

Most Chimera EM users study molecular assemblies with single particle reconstruction (back-projecting thousands of 2-d particle images).

Chimera map visualization tools have been developed for this audience for 6 years with primary collaborator Wah Chiu, director of the National Center for Macromolecular Imaging (NCMI).

Chimera map capabilities are described in Guide to Volume Data Display. Display, fitting, carving, and measuring maps and handling large multimeric atomic models (viruses, ribosomes, filaments, ...).

Estimate 500-1000 EM Chimera users based on email support requests from ~100 distinct users per year.

Estimate 50-100 EM tomography users based on email correspondence.
Why add EM tomography capabilities to Chimera?

Chimera has been used for exploring EM tomograms (calculated from tilt series of single specimens) although we did not develop tools specifically for this use until this year.

Strong encouragement from collaborators

- Manfred Auer (LBNL, stereociliar bundles)
- Wah Chiu (National Center for Macromolecular Imaging),
- Wolfgang Baumeister (MPI, cellular organization)
- Yifan Cheng (UCSF, clathrin vesicles)
- John Sedat (UCSF, drosophila chromosome structure)
- Gary Ren (UCSF, low density lipoprotein cholesterol packaging)

Growing literature of tomography applications to molecule-scale architecture, where Chimera map and atomic model capabilities have been used.
Molecular-scale EM Tomography Examples

Some published examples using Chimera for EM tomography (all completed before we added specific tomography support).

**Clathrin vesicles, Yifan Cheng.**

Cheng Y, Boll W, Kirchhausen T, Harrison SC, Walz T.
Cryo-electron tomography of clathrin-coated vesicles: structural implications for coat assembly.

In vivo clathrin cages have many topologies. Modeling a cage from tomogram. In vitro prepared clathrin allows averaging.

**Stereocilia molecular linkers** (unpublished), *Manfred Auer* lab.

Traced links composed of a few molecules. Scale bar 100 nm.

SEM of hair bundle Tomogram section of tip links.
**HIV glycoprotein spikes, Roux lab.**

Distribution and three-dimensional structure of AIDS virus envelope spikes.  

**Carboxysomes, Michael Schmid** (co-director of NCMI).

Schmid MF, Paredes AM, Khant HA, Soyer F, Aldrich HC, Chiu W, Shively JM.  
Structure of Halothiobacillus neapolitanus carboxysomes by cryo-electron tomography.  
*J Mol Biol.* 2006 Dec 1;364(3):526-35.

**Herpes virus portal, Wah Chiu lab.**

Chang JT, Schmid MF, Rixon FJ, Chiu W.  
Electron cryotomography reveals the portal in the herpesvirus capsid.  
Demonstration of Tomography Capabilities

Example: Human cytotoxic T-cell killing neighboring cell.


Displaying segmentations. Can read IMOD segmentation files, hide, show, split, color, measure enclosed volumes (with holes) and surface areas of objects.

IMOD segmentation of tomogram: lysosomes (blue), microtubules (red), centriole (yellow), golgi (green), cell boundary (orange).

**Single plane display** is most effective view due to high noise level. Contour surfaces, the common display style for single particle reconstructions, are much less useful.
**Resampling.** Extract subregion with axes rotated relative to full data. Interesting structures are generally not aligned with tomogram axes.

**Masking.** Extract density bounded by IMOD segmentation surfaces.

Mask data within selected lytic granule.

Volume masked above and below cell interface.

**Slice plane at any orientation.**

Nuclear pores.

Density in plane of one pore (blue).
New Developments for Single Particle Reconstructions

Masking capability for tomography is also useful for single particle reconstructions.

![Phage K1E.](image)

Contour surface of Gaussian smoothed data separates protein capsid from DNA genome.

![Masked DNA shows spiral packaging.](image)

Morph Map

New morph map tool linear interpolates between maps. Effective method for viewing differences between related maps. Most EM Databank maps belong to sets of related maps.

Original Chimera code contributed by Wei Zhang from Pawel Penzcek's lab.

Two conformations of human RNA polymerase II.
Clash Detection While Fitting

New detect clashes tools shows steric clashes as yellow lines while model is interatively hand-fit or locally optimized.

Symmetry copy command makes copies of contacting monomers.

Fitting alpha and beta tubulin into a microtubule map.
Promoting Chimera in the EM Community: Meetings and Journal Articles

We promote Chimera through demonstrations and hands-on tutorials at meetings:

- Biophysical Society annual meeting, Baltimore, March 5-6, 2007, vendor booth presentation.
- EMAN workshop, Baylor College of Medicine, March 14-17, 2007, Chimera tutorial, ~50 participants.
- Bay Area cryoEM Meeting, UC Davis, May 4, 2007, Chimera tutorial and demo
- Workshop on Advanced Topics in EM Structure Determination, UCSD, Nov 14, 2007, Chimera demonstration

and through publications.


Focus areas for next year

1) Masks for density maps: file formats, creating and applying masks.
   - Can use map files with 0/1 values, 0 to N values, or bit masks for overlapping segments.
   - Allow user naming of segments, and user interface to show/hide/color any set of segments.
   - Create masks with watershed, volume painting (extending volume eraser).
   - Tools that work on full map (fitting, filtering, calculating sdev, segmenting) should be usable on individual segments.

2) Tomography filters: bilateral, median, anisotropic diffusion.
   - These are popular algorithms for denoising tomography data. Chimera already has Gaussian filtering.
   - Allow filtering on small subregions for user to compare filters and determine best parameters interactively.
   - Desirable for filters to run in separate thread with progress report and ability to cancel.

3) Seamless user interface for atomic models of molecular assemblies.
   - Reduce hurdles to using PDB biological unit coordinates.
   - Many PDB entries (32%) do not contain coordinates for the biological unit.
   - Chimera currently has separate user interface for creating, show/hide, selecting, coloring, styles, ... of quaternary structure.
   - Incorporate multimer handling into standard Chimera Actions / Selection / command user interface.
   - Support multiple assembly specifications in mmCIF.
Beyond next year: Animations and EM Databank

Four software development areas of our molecular assemblies NCRR core project:

- Single particle reconstructions.
  - Data standards for EM maps, masks, and meta-data are being advanced as part of merging EM Databank into wwPDB.
  - We are advising and testing proposed standards (HDF maps).
  - Will have large impact on interoperability of software and reuse of archived data.
- Animations of assemblies.
  - Current animation support is piecemeal, e.g. morph map has its own movie recording option.
  - Future plan is for graphical animation editor and suite of commands for common animation transitions (movement, fade in/out, recolor, morph atomic models and maps, map slicing, ...).
- EM tomography.
- Atomic models of large molecular assemblies.