Segmentation and Measurement Methods for Bacteria in Termite Hindgut

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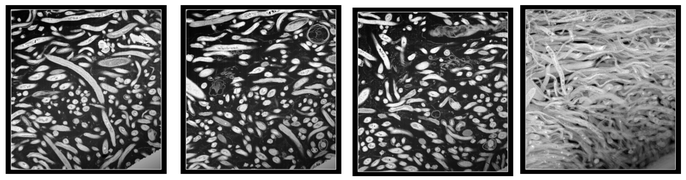
A meta-genomics study suggests there are 200 species of bacteria in the hindgut of termite *Nasutitermes corniger* where lignocellulose plant material is degraded. Some of these bacteria may have value for biofuel production. The cells are worm-shaped and tightly packed like a plate of spaghetti, each cell touching approximately 30 adjacent cells. We present new segmentation and measurement methods to analyze bacterial cells in termite hindgut imaged by focused ion beam scanning electron microscopy (FIBSEM). The segmentation method flood-fills watershed regions using mouse clicks and drags. About 500 cells can be segmented in one day. We developed approaches to compute cell length and diameter and unbend and show curved sections of worm-shaped objects. The software is part of the free UCSF Chimera visualization program (www.cgl.ucsf.edu/chimera).

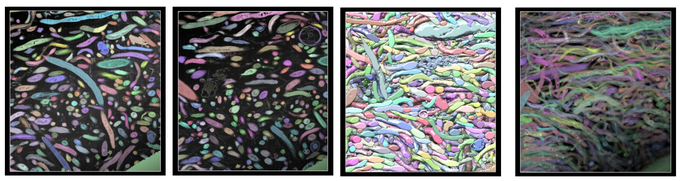
Microbial Community

Map name: LBL_09-2009
File name: sem1_flat.cmap

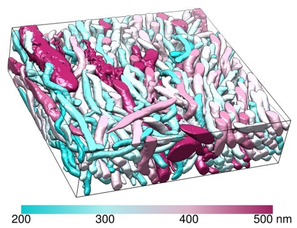
Grid size: 9 x 9.6 x 2.4 um (1808,1483,161)
Voxel size: 5 x 6.5 x 15 nm
Number of segmented cells: 574



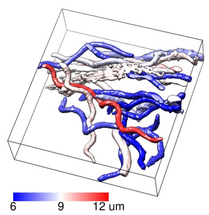
Plane 0. Plane 80. Plane 160. All planes.



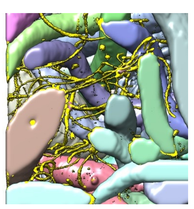
Plane 0 colored. Plane 80 colored. Cell surfaces. All planes colored.



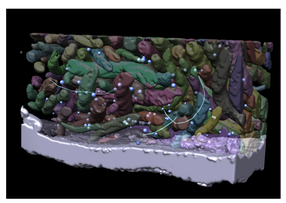
Coloring by cell diameter.



Longest cells colored by length.

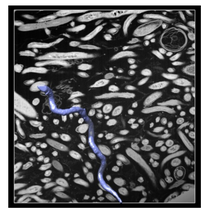


Filaments (flagella?) attached to cells.

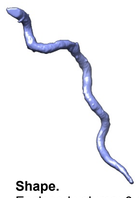


Intercellular vesicles and traced filaments.

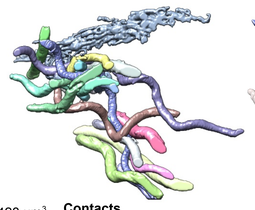
Viewing and Measuring Cells



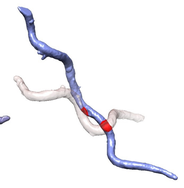
Position.
Grid bounds: 232,0,56 to 1076,900,158
Truncated: yes



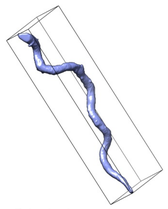
Shape.
Enclosed volume: 0.428 um^3
Surface area: 8.53 um^2



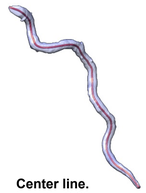
Contacts.
Number of contacting cells: 29



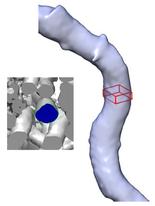
Contact area: 0.264 um^2



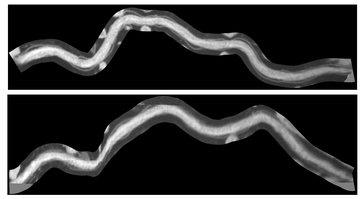
Principal axes box.
Size: 7.20, 2.03, 1.25 um



Center line.
Length: 9.00 um
Ave curvature: 1.55 um^{-1}
Max curvature: 4.22 um^{-1}
Min curvature: 0.172 um^{-1}

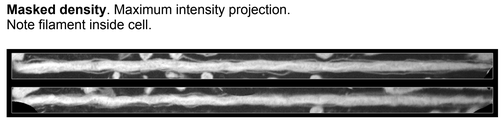


Cross-section.
Diameter: 260 nm
Diameter perp: 219 nm

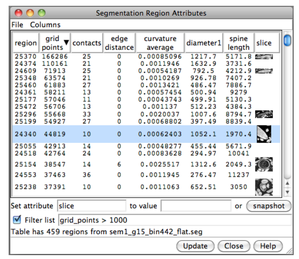


Center slices. Curved ribbons 500 nm wide following center line.
Two orthogonal 3-dimensional ribbons.
Note indentations of envelope by contacting cells.
Enveloped cell: yes

Masked density. Maximum intensity projection.
Note filament inside cell.

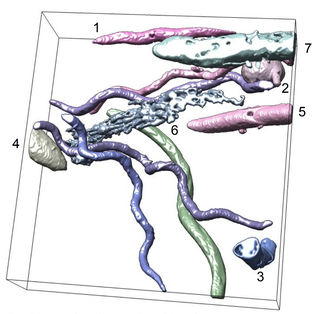


Straightened cell. Orthogonal central slices.

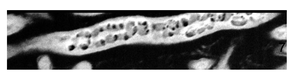


Attribute Table. Measurement values, images, and notes displayed in a table and saved in HDF5 format segmentation files.

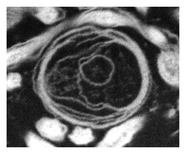
Diverse Cell Morphologies



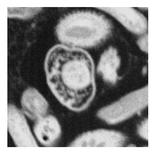
Positions of gallery cells with a few common cells for reference.



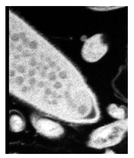
1. Holey



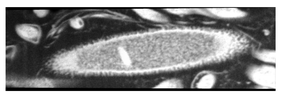
2. Onion



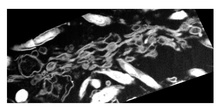
3. Egg



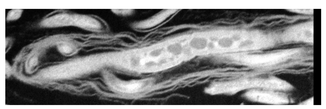
4. Dotty



5. Fuzzy



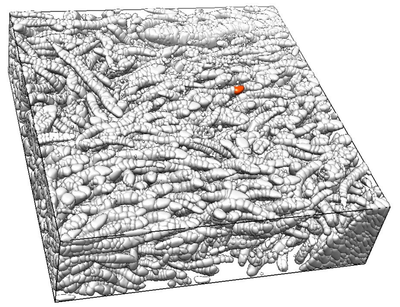
6. Melting



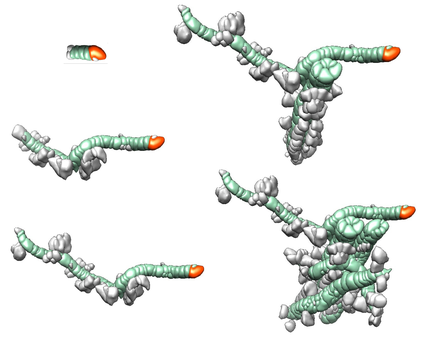
7. Croissant

Central slices of 100 straightened cells.

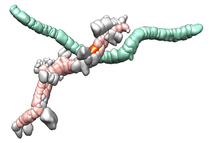
Segmentation Method



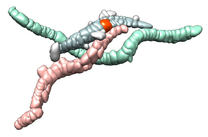
Watershed regions around each local maxima of smoothed and binned data (15 nm Gaussian smoothing, 4x4x2 binning).



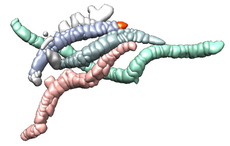
Mouse click and drag up or down to grow or shrink a group of regions composing a cell. Orange shows location of initial click.



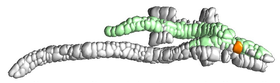
Each click and drag makes a group for a new cell with a new color.



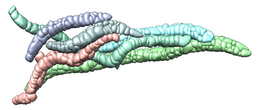
New groups will not grow into previously colored regions.



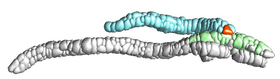
Ungrouped neighbors are shown for the last completed cell.



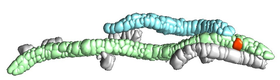
Segmenting sometimes joins two contacting cells instead of forming a single cell.



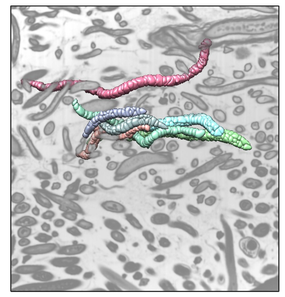
To segment both cells, first color part of one cell near the area of contact.



Next color all of the second cell which will not spill into the already partially colored cell.



Finally, click and drag on the partially colored cell to complete that cell. Three click and drag operations are used to color the two cells.



Click on a cell in the density map and drag to segment it. This is a second way to choose cells, clicking on watershed surfaces is the first way.

Hard Problems

1. Multi-resolution segmentation, subcellular structures.
2. Tedious segmentation process. Use topology hints, e.g. no branching.
3. Slow interaction with large data sets. Optimize code.
4. Web interface to EM segmentation results. Database.
5. Segmentation file format, HDF5 used in Chimera.

Obtaining Software

Google **Chimera.** Go to download page.
See video documentation and volume guide.

[1] Resource for Biocomputing, Visualization and Informatics, UC San Francisco.
[2] Lawrence Berkeley National Laboratory.
[3] National Center for Macromolecular Imaging, Baylor College of Medicine.