Segger – Icosahedral Segmentation (iSeg)

Last updated: May. 30, 2019 (Segger v2.3)

Overview

In this tutorial, we will use the iSeg tool to segment the coat, DNA layers, and portal-hub-adhesin complex in an ichosahedral virus.

For this tutorial, download the density map for P22 virion, EMDB:8005.

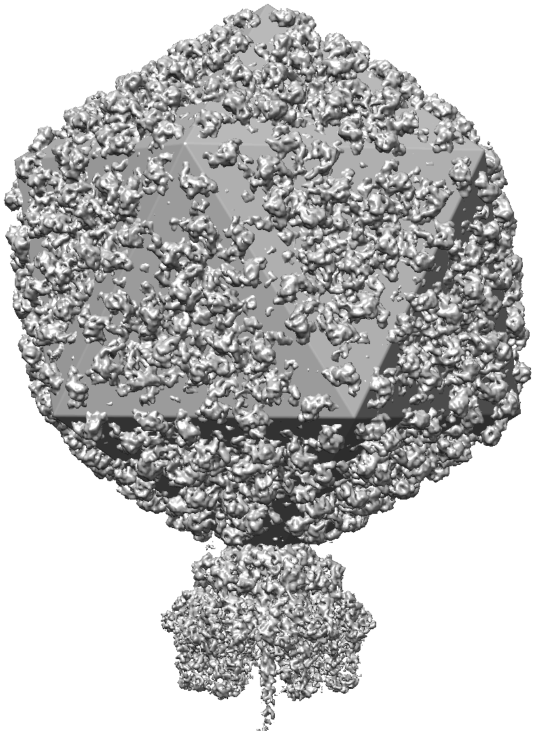
1. Opening the map and Fitting an Icosahedron to it

- Open the map EMDB_8005, and set contour level to about .2
- Open Tools -> Higher Order Structure -> Icosahedron Surface
  - Set radius to 345
  - Set Orientation to … (n25r)
  - Set Subdivision factor to 100, Surface style to solid.
  - Press the Show button at the bottom of the dialog.

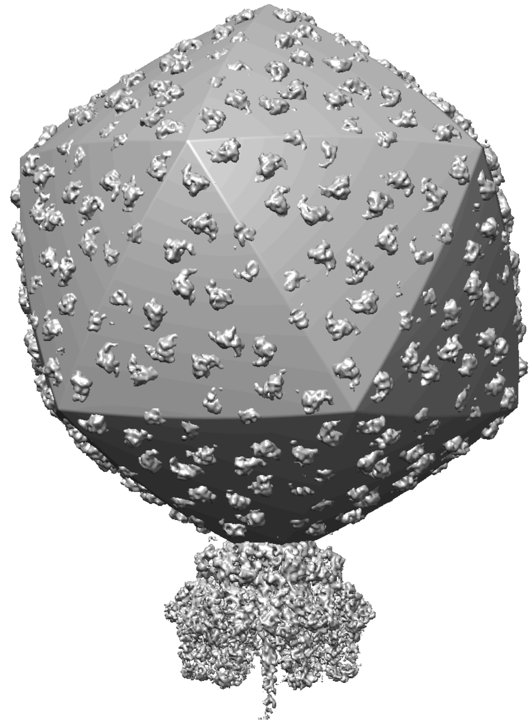
You should see the image on the left below. Notice how the map seems to bulge out in the middle of each icosahedral triangular face. We can try to see if the map is more like a sphere…

- In the Icosahedron Surface dialog
  - adjust Sphere factor to ~0.3.

Now the Icosahedron looks like in the image on the right, below. This seems to fit the map better, but the icosahedral triangle edges are also bulging, which doesn't seem to match the map that well. (This is even more apparent in larger viruses than this one, e.g. CIV, SGIV).



Icosahedron shown on top of P22 map.

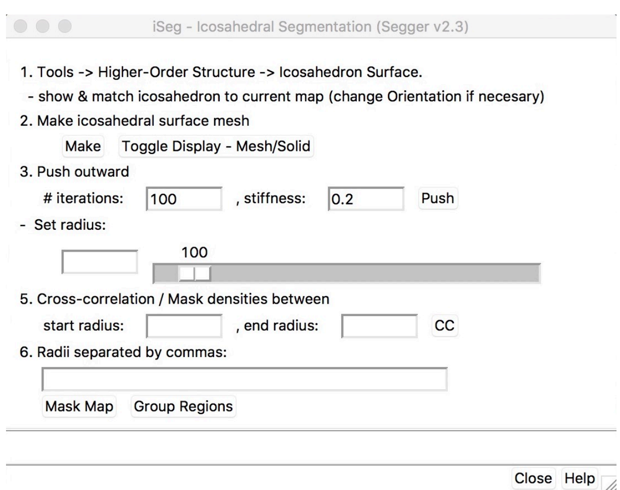


Icosahedron with sphere factor ~0.3.

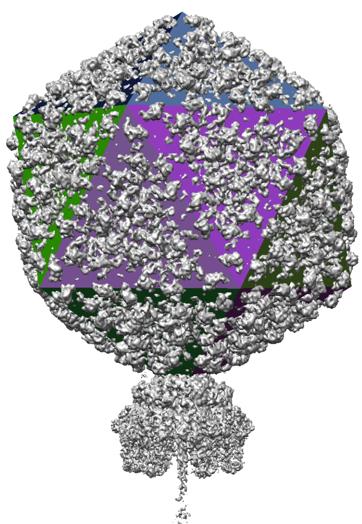
2. Using iSeg

- In the Icosahedron Surface Dialog:
    - Set Subdivion Factor to 1
- Open Segment Map dialog (Tools -> Volume Data -> Segment Map)
    - Press the iSeg button.

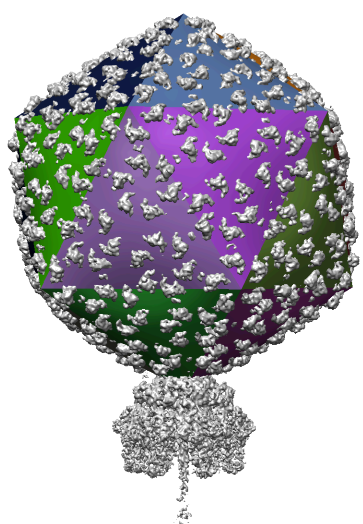
You should see the iSeg dialog, as below:



- In the iSeg Dialog:
  - Press the Make button under 2.
    - You can toggle display to switch between Mesh and Solid. The Solid looks like below on the left.
  - Now let's push on the icosahedron
    - Want to make it bulge like the map, but keeping the icosahedral triangle edges fixed.
    - You can try different parameters (# iterations and stiffness).
      - The stiffness controls how stiff the surface is. Higher numbers make the surface stiffer, and hence bulge less.
      - You should choose a high number of iterations so the geometry equilibrates under the pushing force, but the more iterations, the longer this process will take.
    - The result for 500 iterations and stiffness of 0.2 is shown below in the middle.



Icosahedral geometry. Every triangular face is a different color.



After pushing on the icosahedron, keeping the icosahedral triangle edges fixed. Note only the middles of the triangles bulge out.

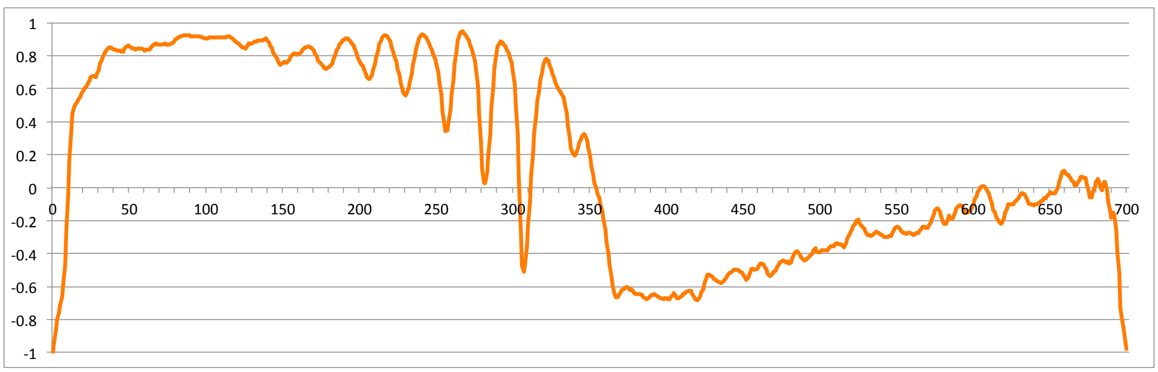
Using the 'Sphere factor' of 0.3 as before. The edges of the triangles bulge too.

- In the iSeg Dialog:
  - Note you can then change the radius of the new bulging icosahedron to different values.

- The shape stays the same; it's only scaled so the radius changes. The radius is taken as the distance to one of the 5-fold axes.
- This 'scaling' of the radius is used to calculate cross-correlation at different radii, and also for masking and segmentation, as described below.

- To calculate cross-correlation between the bulging icosahedron and the density map:
  - Open a map and select it in the Segment Map dialog 'Segment Map' field.
  - Start radius: 0
  - End radius: 700
  - Press the CC button
- When done, a 'Save file' dialog will appear. Select location and name of file to write the radius/cc values to.

➢ Open/plot the radius/cc values with your favourite plotting program. The plot looks as below.



Note the peaks, which correspond to layers in the packaged DNA. The final peak corresponds to the coat proteins. The drop after and small gradual rise corresponds to the tail. Note also that you can repeat the process with different # iterations and stiffness with your data, to see if you get better peaks – the closer the icosahedral geometry matches the map, the better peaks you will get.

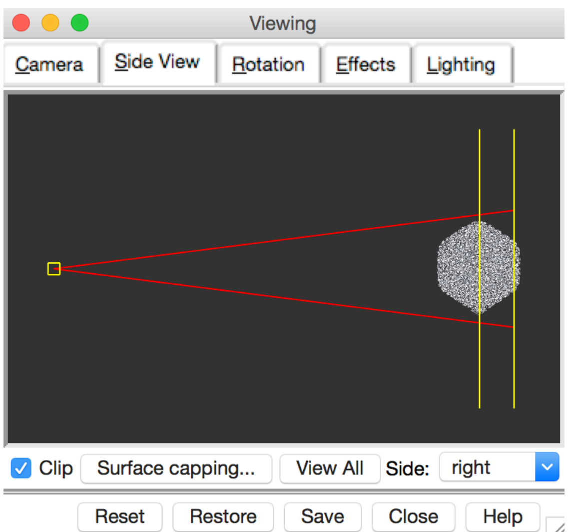
To extract a single layer from the map, e.g. the coat:

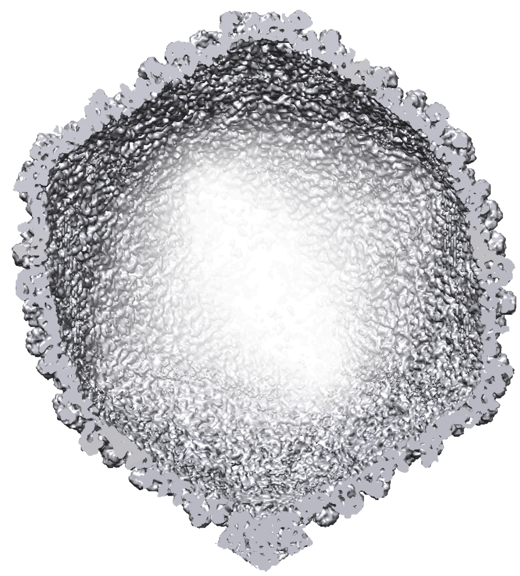
➢ In the iSeg dialog
  - Enter the values on either side of the peak in the text edit under "6. Radii separated by commas", e.g. 300,370
  - Press the "Mask Map" button.

➢ Note, this process can take a long time…
  - There are a lot of grid points to check in large maps
    - In this one 512*512*512= 134,217,728 grid points.
    - on a i7 this takes about 1 hour

    - For each grid point, its position is checked whether it's outside the start radius, but inside the end radius; this is a costly operation given the complex bulging icosahedron
    - The code is in written python (the language extensions to Chimera can be written in), which can be ~100 slower than compiled C code. Also it runs only on single thread.
- The process below, using segmentation, is much quicker
    - There are fewer regions to check than grid points (same check is made for each region).
    - You can get a 'masked' map just as here from a resulting region using the 'Extract' dialog. (See the Extract tutorial).

To see the masked shell, the clipping plane can be adjusted in the side view (Tools -> Viewing Controls -> Side View):

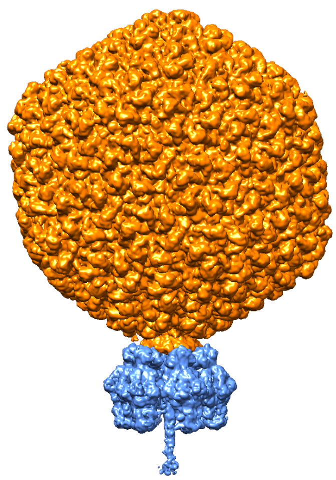




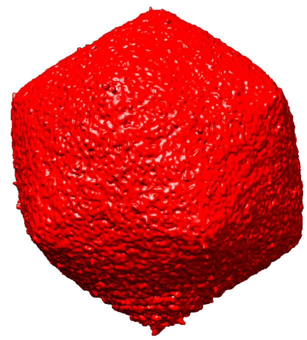
To segment all layers:
- In the Segment Map dialog
    - select the map in Segment Map field
    - set smoothing steps to 0 (press the Options button at the bottom first)
    - press the Segment button
        - This will create 102,374 regions at a threshold of 0.1
        - Not all regions will be shown, just the largest 2,000 by default
        - These regions can now be grouped based on radii entered in the iSeg dialog.
- In the iSeg dialog
    - Enter the values corresponding to valleys between the peaks in the plot above:
        - 146,176,203,228,255,281,306,370

- Press the “Group Regions” button.
  - This will take about a minute or so on this map, you can watch the progress in the status bar.

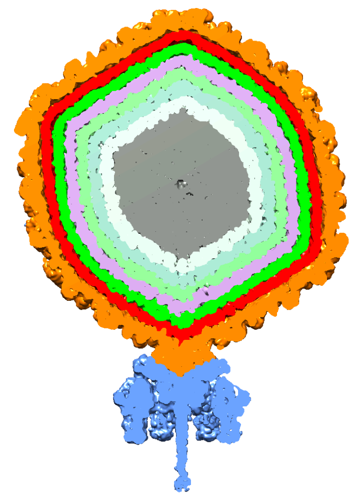
When done, 9 regions will have been created, as shown below:



The outermost regions after grouping based on radial distance, with adjustment for *bulging* icosahedral geometry.

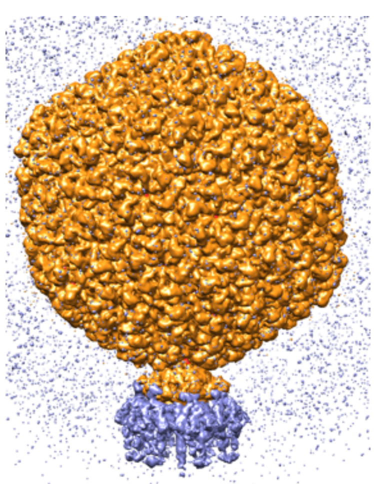


The 3rd layer after hiding the outermost 1.

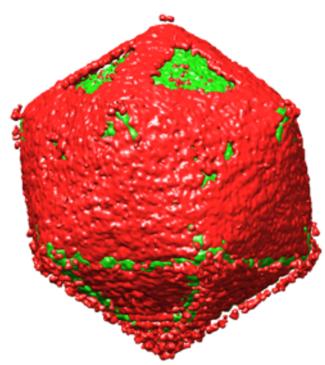


All layers shown by clipping through middle.

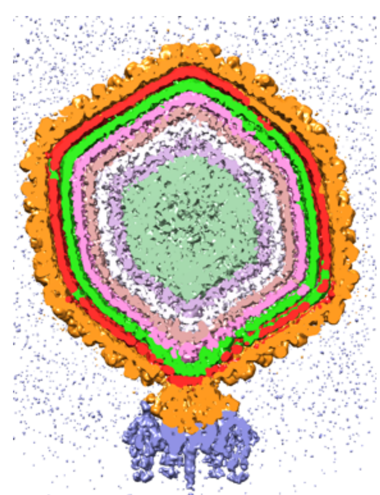
Better than the rSeg results shown below! (Note the map used in that tutorial has noise outside the capsid, the map used here does not).



The outermost regions after grouping based on radial distance, with adjustment



The 3rd layer after hiding the outermost 2.



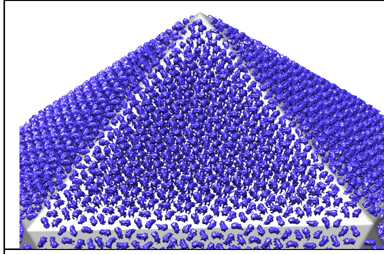
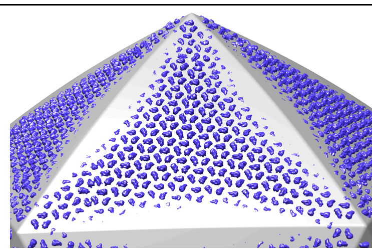
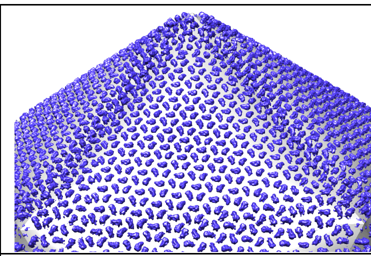
All layers shown by clipping through middle.

for icosahedral geometry.

Note that for very large maps, (on the order of gigs), Chimera may crash when segmenting or masking as outlined above. In this case, one possible way to tackle it would be:

- center the map (Volume Viewer -> Features -> Coordinates -> center), and
- crop a part of it (in the Volume Viewer dialog, use Features -> Subregion Selection)
- save the cropped map
- open the cropped map as a new file
- do the process above on the cropped map

Example with a larger map (SGIV / EMD 1580):

		
Icosahedron (gray) fitted to map (blue).	Icosahedron with sphere factor 0.2.	Icosahedron pushed with iSeg.