Segger – Radial Segmentation (rSeg)

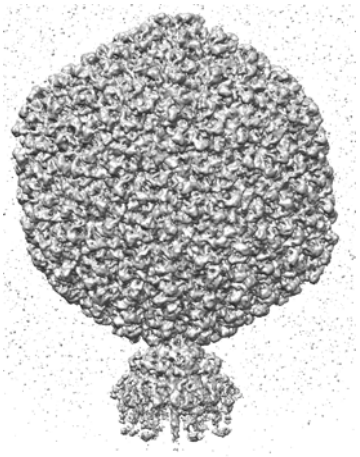
Last updated: Oct. 24, 2015 (Segger v1.9.2, Chimera Version 1.11.0)

Overview

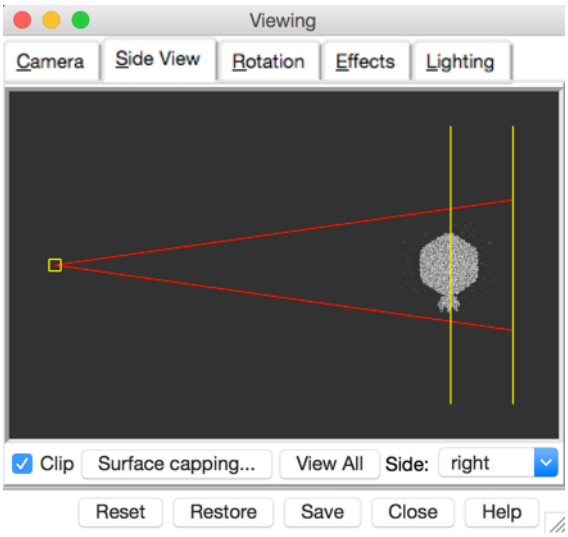
In this tutorial, we will use the rSeg tool to segment the coat and DNA layers in an ichosahedral virus.

For this tutorial, download the density map for P22 virion from this link.

Open the map in Chimera, and set the threshold in the Volume Viewer dialog to 0.2. Turning the virus ~90° around x-axis should look like the image below.



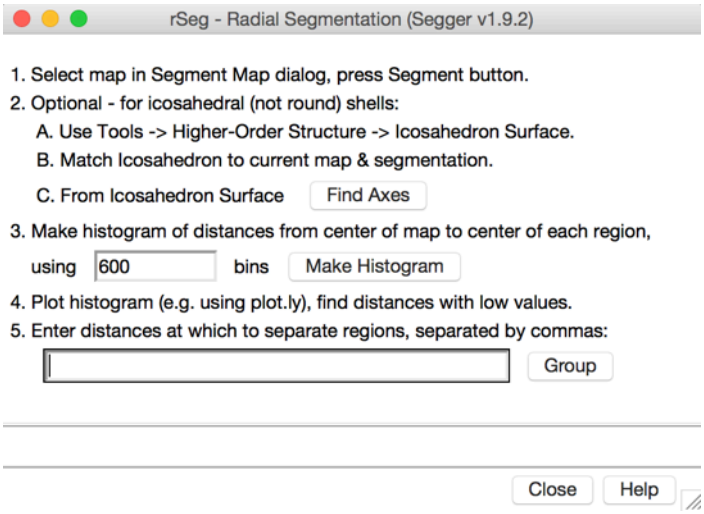
To see the inside of the virus, open Tools -> Viewing Controls -> Side View, and move the yellow line on the left to the middle of the virion, as such:



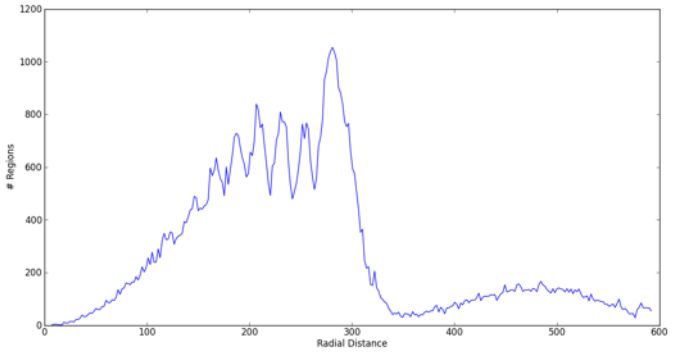
The icosahedral capsid can clearly be seen in the slice, and moreover the DNA layers inside themselves follow icosahedral geometry especially close to the capsid.

To segment the capsid and the DNA layers, we can first try radial segmentation, and see how well it does.

- Open the Segment Map dialog from Tools -> Volume Data, and select the map in the 'Segment map' field at the top.
- Then press the **Options** button at the bottom of the dialog.
- Enter 0 for 'Smoothing steps'.
- Because clipping can confuse Segger as it keeps track of regions surfaces, also press **View all** at the bottom of the Viewing dialog (above picture on the left).
- Press **Segment** at the bottom of the Segment Map dialog.
    - Note that this will produce ~80,000 regions, but Segger will not display them all, as that would slow down the display immensely.
- Press the **Shortcuts** button on the Segment Map dialog, and then press **rSeg** to the right of Other tools. This will bring up the rSeg Dialog:



- We've already done step 1, and for now we will not account of icosahedral geometry, so let's go on to step 3.
- Set the number of bins according to your best guess. A rule that seems to work well is to set it to $\sqrt{N}$, where N is the number of samples. In this case it's ~80,000, and $\sqrt{80{,}000} \approx 300$, so entering 300 for number of bins works quite well.
- Press the **Make Histogram** button.
  - This will go through the ~80,000 regions and calculate the distance from the center of the map to the center of the region (the radial distance).
  - After the calculation is done, you will see a new dialog asking you to confirm where the histogram data file will be saved. Name it dist_hist_sphere.txt, and place it in the same path as where you uncompressed the downloaded file for this tutorial (path should have name segger_tutorial_rSeg).
  - In terminal cd to that path, and then run the python plot script:
    - `python plot_sphere.py`
  - You should see a plot like this:

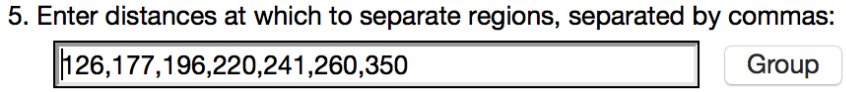


  - Notice how there are obvious peaks and valleys. Moving the mouse over the plot, you'll notice the x and y values reported below the plot.

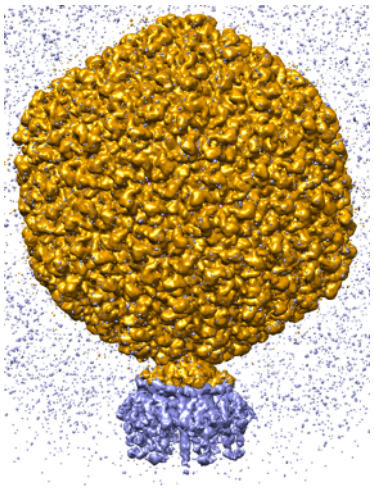
Let's read out where valleys are – this is where there are fewer regions with that radial distance, hence lower density shells:

- 126,177,196,220,241,260,350

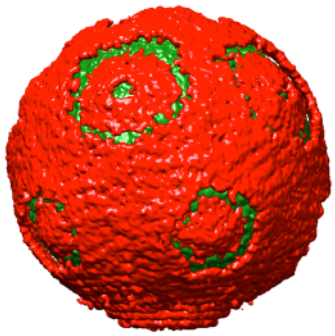
- Enter those numbers as above, with commas:



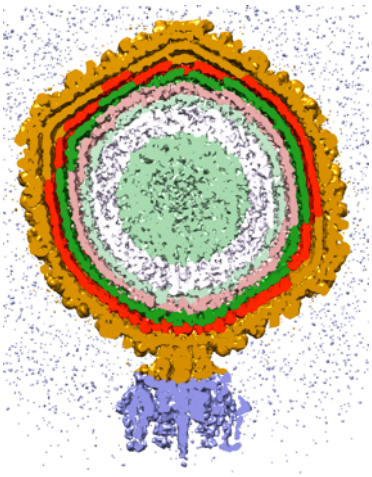
-
- Press the **Group** button.
- Going back to the Segment Dialog, you'll see now there are only 8 regions.
- The outermost layer is some noise outside the capsid and the tail (light blue in below images). Then the next layer is the 'capsid' (brown in the images below).
  - Hide these two layers by ctr+click on them, then Selected Regions: **Hide** in the Shortcuts panel. You'll see the first 'DNA' layer (red in images below).
- Using the clipping plane as above in the Viewing dialog, we can look at the segmented layers, as shown on the right below.



The outermost regions after grouping based on radial distance



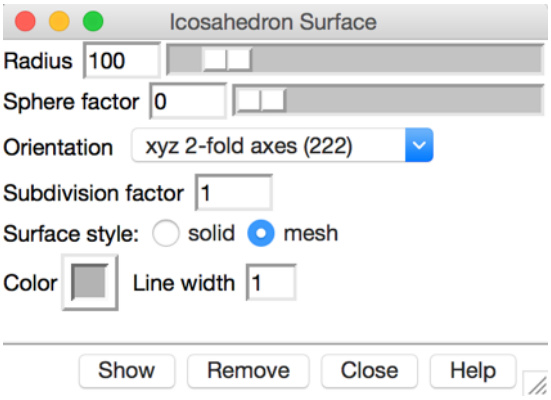
The 3rd layer after hiding the outermost 2.



All layers shown by clipping through middle.

We can see the layers above are not quite icosahedral, so let's correct the radial distances based on icosahedral geometry.

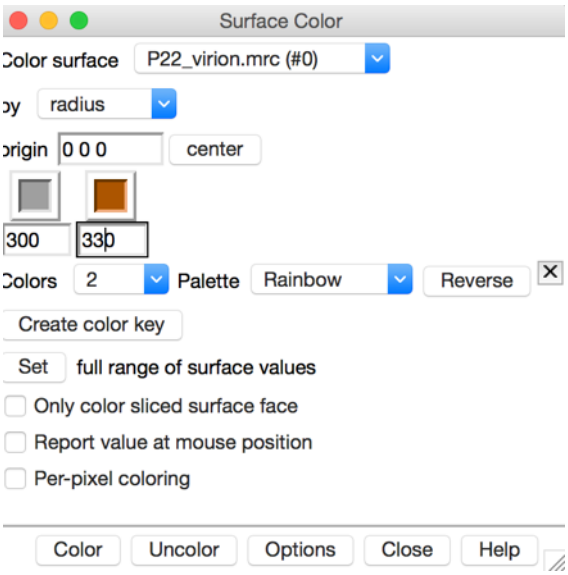
- In Model Panel, show the P22_virion.mrc model.
- Tools -> Higher-Order Structure -> Icosahedron Surface.

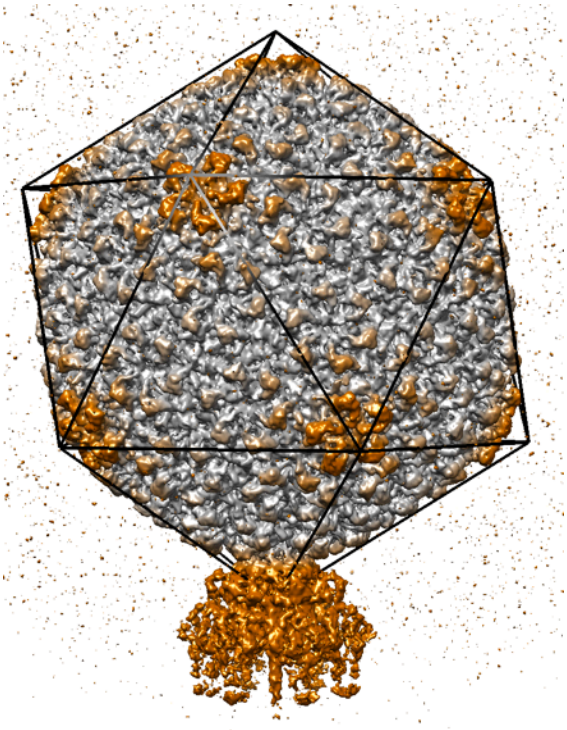


- In the Icosahedron Surface dialog, press the Show button, and then drag the Radius slider until you see the icosahedron wireframe appear, for a Radius of ~400.
- If you look carefully you'll notice the default icosahedron Orientation does not match up well with this map. The correct Orientation is the last entry in the list as below:

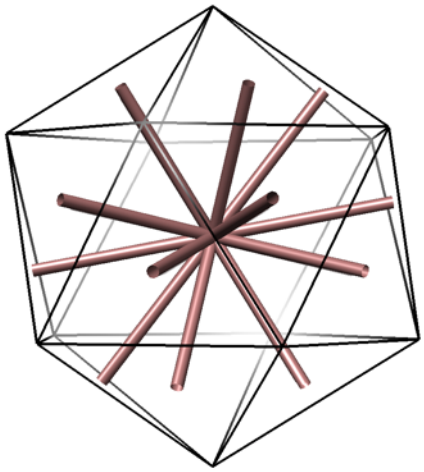
  Orientation y 2-fold, z 5-fold, alt (n25r)

- In that orientation, you'll see the icosahedron aligns well with the 5-fold vertices in the capsid.
  - To better see the 5-fold vertices in the capsid, use Tools -> Volume Data -> Surface Color, and set as shown below on the left.

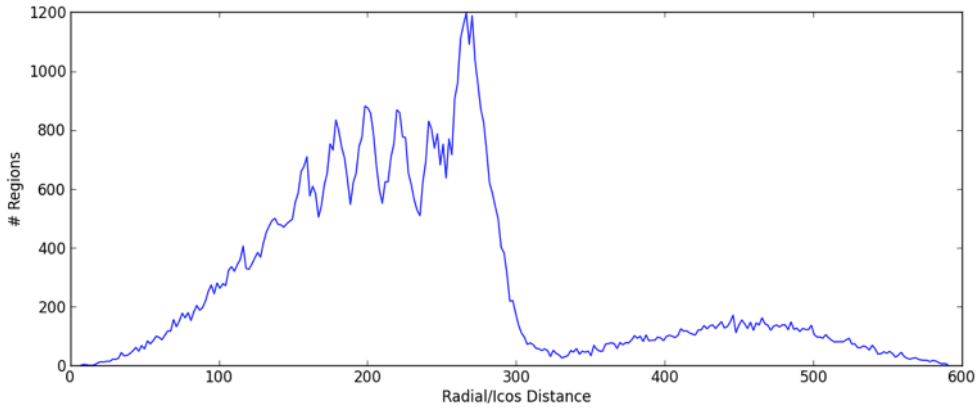




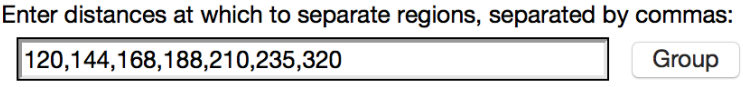
- Now let's use the icosahedral geometry to adjust the radial distances.
- In the rSeg Dialog, press the **Find Axes** button.
    - If you hide the map in Model Panel, you will see the axes as such:



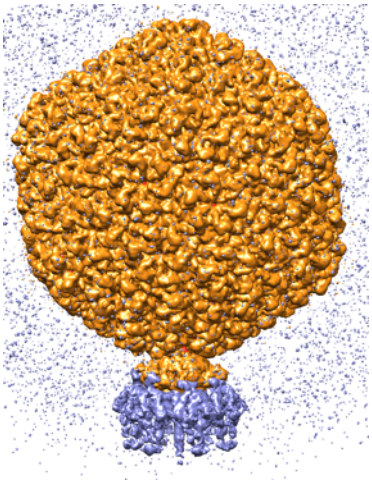
    - These axes go from the center of the icosahedron to the midpoint of each of the 20 triangles that make up the icosahedron.
    - Then press Make Histogram again.
        - Now the radial distances are projected onto the closest axis, which adjusts for the icosahedral geometry.
        - Note the radius of the icosahedron doesn't matter, it's only the direction of the axes that is used.
        - Save the result as 'dist_hist_icos.txt'
        - In the terminal, run
            - `python plot_icos.py`
        - You should see a plot appear:



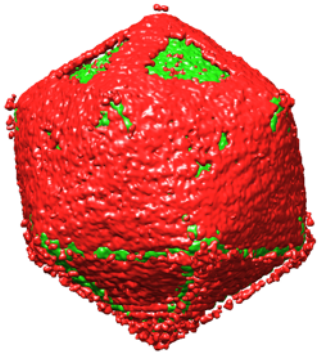
- Find and enter the x-values for the valleys in the rSeg dialog
    - 120,144,168,188,210,235,252,320



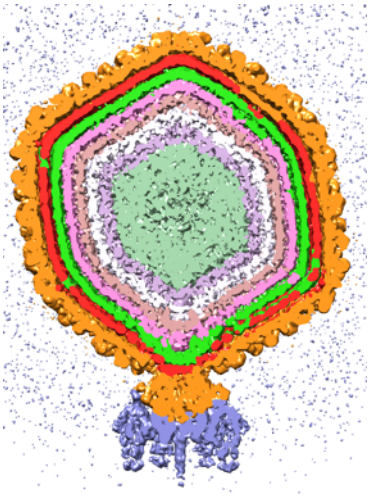
- Then press **Group**.



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

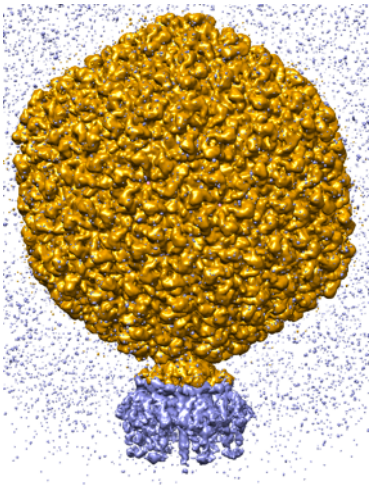


The 3rd layer after hiding the outermost 2.

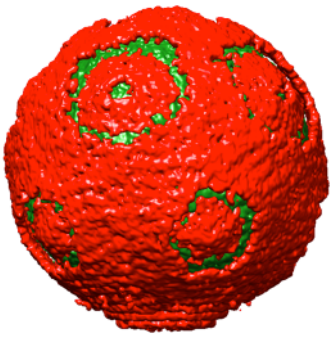


All layers shown by clipping through middle.

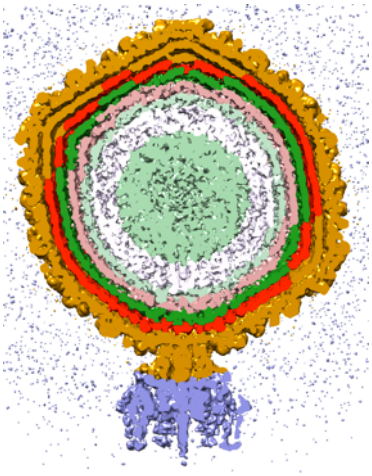
You'll notice that the segmented layers, as shown above, follow the icosahedral geometry much more closely than before. The segmetation based on spherical distance is shown below for comparison.



The outermost regions after grouping based on radial distance



The 3rd layer after hiding the outermost 2.



All layers shown by clipping through middle.

Even with the icosahedron-geometry adjustment, the segmentation is not perfect, as the virion itself in nature is not perfectly icosahedral, but the segmentation can be further adjusted interactively using group/ungroup operations.