Instructions for Segmentation of Icosahedral Virus Maps

The steps to segment each map (I'm using the example emd_1011.map):

1. Run VolRover (you need to download VolRover from CVC webpage). Read the map. Adjust the little cube in the right window to the maximal size. Save the Subvolume as "rawiv" type.

2. In VolRover, you also need to decide visually the *h* and *k* numbers (***h_num*** and ***k_num***) of the virus structure. If you have difficulty in finding these numbers, please check the reference of that map in the above link, where you may find this information. You need to decide if there is a 5-fold subunit (if yes, the parameter ***5-fold*** = *1*; otherwise, 0). You also need to decide whether the major subunits to be segmented are located at the 3-fold grids or 6-fold grids (see Figure 1). If the subunits are at the 3-fold grids (red dots), ***6-fold*** = 0 and ***3-fold*** is equal to the folding number of the subunits. If the subunits are at the 6-fold grids (blue dots), ***3-fold*** = 0 and ***6-fold*** is equal to the folding number of the subunits. Again, reading the reference would be helpful for you to decide these parameters.

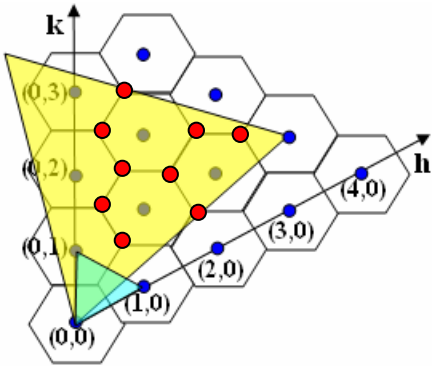

Figure1 The blue dots are 6-fold grids and the red dots are the 3-fold grids.

3. Copy the rawiv map (named *emd_1011.rawiv*, for instance) to your working directory.

4. Compile the *makeslice_z.c* by "*cc makeslice_z.c –o makeslice_z*".

5. Run *makeslice_z* as follows:

makeslice_z emd_1011.rawiv tt.ppm slice_num

where *slice_num* is usually half the dimension in z-axis. If you don't know the dimension, try zero and *makeslice_z* will tell you the dimension of the map. The following figure shows the central slice of *emd_1011.rawiv.*

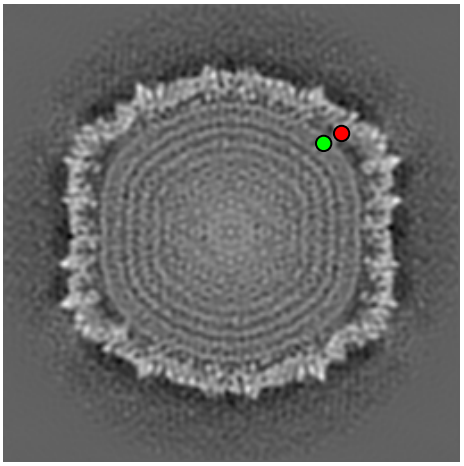

Figure 2

6. Type "xv *tt.ppm*" on maverick and you will see the above figure. Use the middle button of the mouse to select the threshold ***tlow*** for the capsid layer. It is very important to choose a good threshold for correct segmentation of the capsid layer. If the capsid layer is indistinctive from the inner genomic structure, it is necessary to manually select two seed points, one inside the genomic structure and the other inside the capsid. The two seeds are usually close to each other are where the capsid is most indistinctive from the genomic structure.

7. Compile the segmentation source code by typing "./a.make". Run the segmentation using the parameters determined above:

To segment the capsid layer:

./VirusegCapsid emd_1011.rawiv 1 120 184 78 128 189 72 128
(if capsid is obvious, use: "./VirusegCapsid emd_1011.rawiv 0 tlow".
Type "./VirusegCapsid" for help)
The output is emd_1011_capsid.rawiv

To segment the subunits:

./VirusegSubunit emd_1011_capsid.rawiv ***h_num k_num 3-fold 5-fold 6-fold***

The segmentation tool will return the following files:

- emd_1011_capsid _matrix.txt: transformation matrices
- emd_1011_capsid _5f_subunit.rawiv: segmented 5-fold subunit
- emd_1011_capsid _6f_subavg.rawiv: average of segmented 6-fold subunits
- emd_1011_capsid _seg.rawv: color map of segmentation
- emd_1011_capsid _index.rawiv: indexing map of segmentation
- emd_1011_capsid_symmetry_axis.raw: symmetry axes
- emd_1011_capsid_similarity_score.txt: correlation scores
- emd_1011_capsid_6f_axis.txt: returned 6-fold symmetry axes
- emd_1011_5f_axis.txt: returned 5-fold symmetry axes

8. Display/Edit the results:

- Symmetry axes: Run VolRover. Open emd_1011.rawiv. From the menu "Geometry", choose "Load Geometry". Select the file "emd_1011_capsid_symmetry_axis.raw" that the segmentation returned just now. You will see the following picture. Save it.

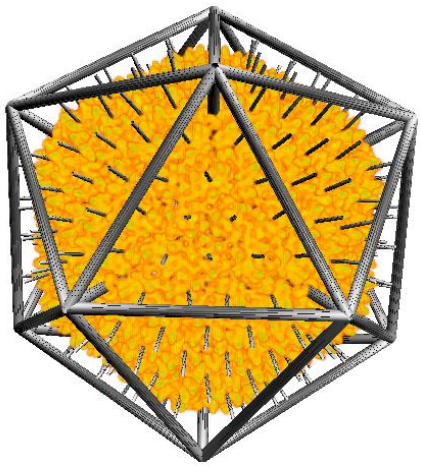

Figure 3.

Important! When making your pictures, please set the background as white (in "File"->"Option"->"Background color"). Disable the wire cube (in "View"->"Show Wire Cube"). Same for the following pictures.

- Segmentation color map: Run VolRover and open "emd_1011_capsid _seg.rawv". From the menu "File", go to "Options.." and select the "RGBA Combined". You will see the following picture (the left one). In VolRover, you can see a few adjustable bars below the left and right windows. Adjust the "Explorer Near Clip Plane" just below the right window. You will see the segmented subunits from the inside (see the picture below on the right-hand side). Save both pictures.

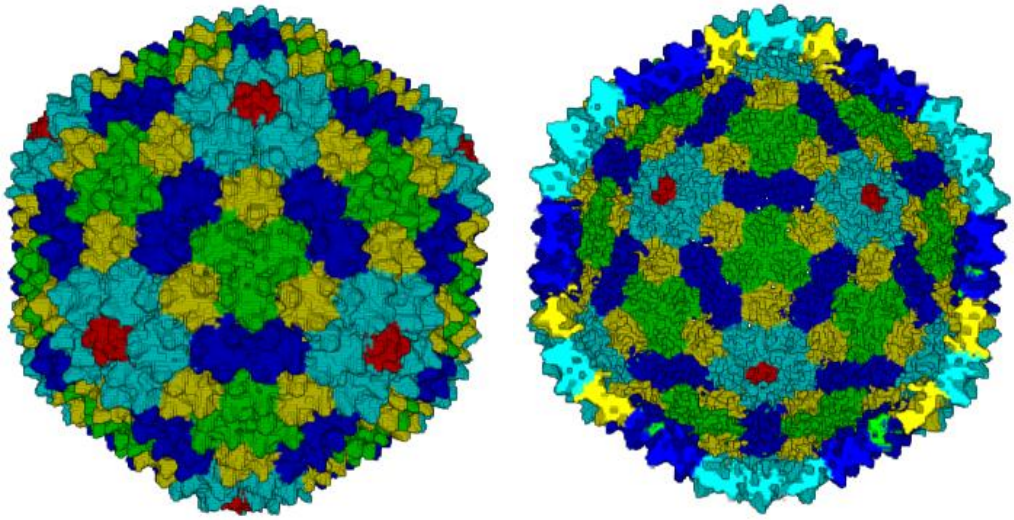

Figure 4

- Segmented 5-fold subunit: Run VolRover and open “emd_1011_capsid _5f_subunit.rawiv”. In the color window (on the bottom of VolRover), Click the right button of mouse, Go to “Add”->”Isocontour Node”. Adjust the node to change the isovalue. Once a good isovalue is selected, save the picture (see example in Figure 5).

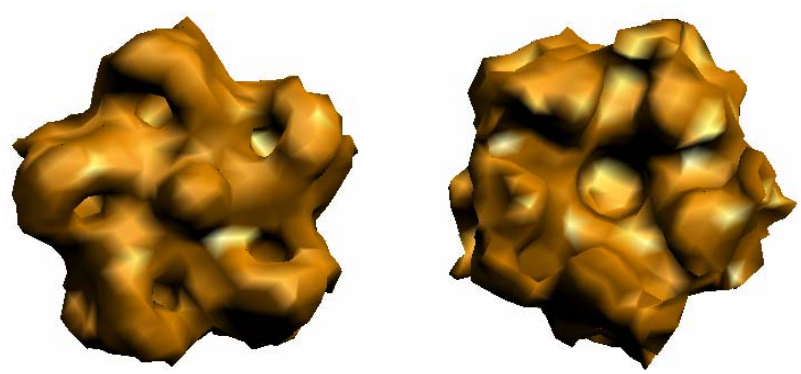

Figure 5

- Average of segmented 6-fold subunits: Run VolRover and open “emd_1011_capsid _6f_subavg.rawiv”. Choose a good isovalue and save the picture (see example in Figure 6).

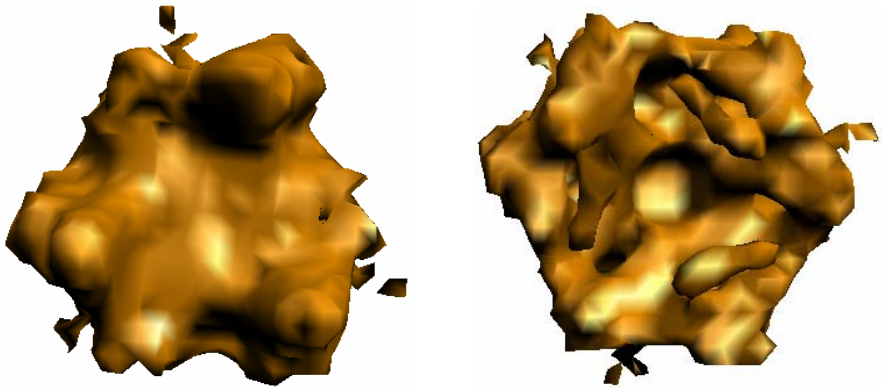

Figure 6

- Similarity Table:
  From “emd_1011_capsid_similarity_score.txt”, we can find the similarity scores between subunits:
  1.000000 0.000000 0.000000 0.000000
  0.887794 -0.800000 3.141592 0.368794
  0.907930 -0.400000 -0.017453 0.190471
  0.889730 -1.100000 1.064651 0.297578

  -1.000000 0.800000 -3.141592 0.368794
  1.000000 0.000000 0.000000 0.000000
  0.899031 0.400000 -3.176499 0.526629
  0.875711 -0.400000 -2.076942 0.201592

-1.000000 0.400000 0.017453 0.190471
-1.000000 -0.400000 3.176499 0.526629
1.000000 0.000000 0.000000 0.000000
0.882088 -0.600000 1.134464 0.391825

-1.000000 1.100000 -1.064651 0.297578
-1.000000 0.400000 2.076942 0.201592
-1.000000 0.600000 -1.134464 0.391825
1.000000 0.000000 0.000000 0.000000

	1	2	3	4
1		0. 887794	0.907930	0.889730
2	0. 887794		0.899031	0.875711
3	0.907930	0.899031		0.882088
4	0.889730	0.875711	0.882088	

From “emd_1011_capsid_6f_axis.txt” (“**_3f_axis.txt”, if the subunits have 3-fold symmetry), we can find the symmetry scores for each subunit (meaning the similarity score between a subunit and its rotated copies):

128.175659 150.750244 228.367996 126.221344 139.842041 189.933731 **0.940744**
…… (59 more)
135.938095 125.366867 233.145020 138.974777 128.185486 193.360168 **0.909652**
…… (59 more)
152.750320 152.618988 227.991394 144.223923 138.450409 191.569519 **0.936145**
…… (59 more)
161.008606 126.777542 233.774170 156.319061 127.336990 194.053955 **0.907511**
…… (59 more)

	1	2	3	4
1	0.940744			
2		0.909652		
3			0.936145	
4				0.907511

Merge the above two tables, we can have the similarity table:

	1	2	3	4
1	0.940744	0. 887794	0.907930	0.889730
2	0. 887794	0.909652	0.899031	0.875711
3	0.907930	0.899031	0.936145	0.882088
4	0.889730	0.875711	0.882088	0.907511

Table 1

Questions? Email to Zeyun Yu: zeyun.yu@gmail.com