

Directional Resolution of Cryo-EM Maps – Using the 3DFSC Server

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Requirements: Web browser (To download datasets)
UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) for visualization

Instructions for Practical

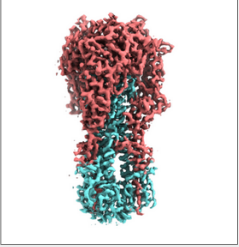
Map Validation – Local Resolution (Directional FSC)

1. Maps usually have variations in resolution, both in real space and in Fourier space. Variation of resolution in Fourier Space can be due to preferred orientation issues
2. To explore directional anisotropy in resolution, go to this online server: <https://3dfsc.salk.edu>
 - a. Register for an account and login
3. Start a new job with another EMDB entry

EMDB > EMD-0152

RELION-3.0 reconstruction of influenza hemagglutinin (HA) trimer using particles from micrographs tilted at 40 degrees in EMPIAR-10097
Source organism: *Homo sapiens* [9606]
Related EM entries by publication: [EMD-0144](#), [EMD-0153](#), [EMD-0263](#)
3Dblionotes: available for this entry
Primary publication:
New tools for automated high-resolution cryo-EM structure determination in RELION-3.
Zivanov J, Nakane T, Forsberg BO, Kimanius D, Hagen WJ, Lindahl E, Scheres SH
Elife 7 - (2018)
PMID: [30412051](#)

Single particle reconstruction
3.10945Å resolution
Map released: 2018-08-08
Last modified: 2018-12-26



b.

Remote 3DFSC Processing Server

Required Fields	Optional Fields
Jobname <input type="text" value="Relion3HA"/>	Mask file <input type="button" value="Browse..."/> emd_0152_msk_1.map
Apix <input type="text" value="1.31"/>	Cone angle <input type="text" value="20"/>
Halfmap1file <input type="button" value="Browse..."/> emd_0152_half_map_1.map	FSC Cutoff <input type="text" value="0.143"/>
Half-map 2 file <input type="button" value="Browse..."/> emd_0152_half_map_2.map	Sphericity Threshold <input type="text" value="0.5"/>
Full map file <input type="button" value="Browse..."/> emd_0152.map	High-pass filter (Angstrom) <input type="text" value="150"/>
<input type="button" value="Submit Job"/> <input type="button" value="Reset Form"/>	

c.

4. Submit the job. It will take about 1 minute to run, but if there is a queue you might have to wait a bit longer
5. Once it is done, navigate to Jobs > Link for the results

Remote 3DFSC Processing Server

Active and completed jobs				
Job ID	Job Name	Job Status	Last Modified	Results
1107	IDetest	SUCCESS	04/16/2019 20:04:12	Link
1106	Relion3HA	SUCCESS	04/16/2019 20:04:53	Link

d.

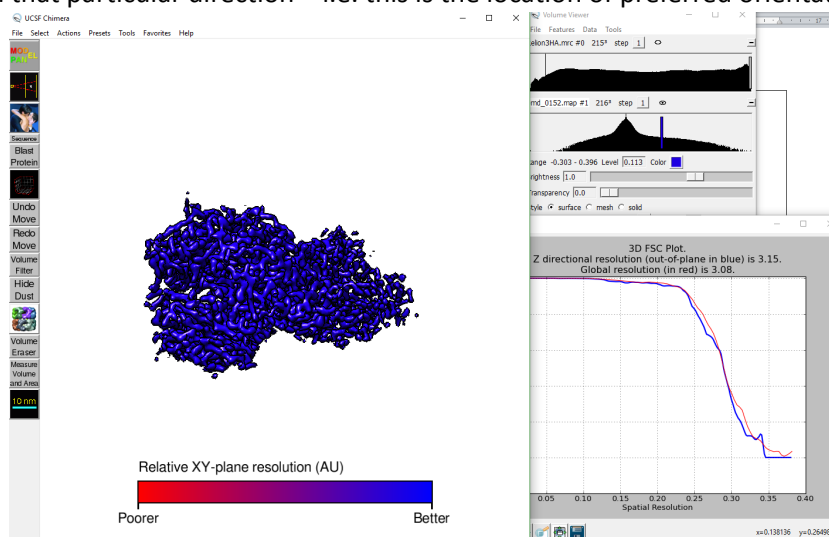
6. Answer the following questions based on your results

What is the sphericity value?

Sphericity indicates how isotropic the 3DFSC is. If you do not have preferred orientation, your 3DFSC is a perfect sphere and the sphericity is 1. Values from 0.9 to 1.0 indicate good sphericity, while values from 0.8 to 0.9 are acceptable. Values below that are usually map plagued by severe preferred orientation.

- Now download the (compressed) results onto your computer. When unzipping the files, make sure you have the password copied.
- Once you have download the results, open another Chimera session. Now go to File > Open and open the file called "3DFSCPlot_Chimera". This will automatically open up the relevant maps in Chimera for you to view the directional resolution of the map
- Move your map around and notice how the color changes. The more blue the map is, the better the XY-plane resolution the view has. This also corresponds (roughly) to having more views in that particular direction – i.e. this is the location of preferred orientation.

e.



10. Answer the following questions.

Which is the preferred view of the protein?

What is the Z directional resolution of the best view?

What is the Z directional resolution of the worst view?	
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