RELION-2 Workshop – 2017-03

Data

Data: Wong et al., Plasmodium falciparum 80S ribosome, eLife 2014
Contaminated with “unknown ribosome”
Publicly available: EMPIAR-10028, www.ebi.ac.uk/pdbe/emdb/empiar/entry/10028
Downsized: original pixel-size = 1.34, workshop pixel size = 2.08

Part 1

1. **Import micrographs**
2. **NOTES: Motion correction**
3. **CTF,**
    1. IO, input imported micrographs
    2. Magnification pixel size = 2.08
    3. Use Gctf instead of CTFFIND = yes
    4. Gctf executable = gctf
4. **Manual picking**
    1. IO, input CTF-job output
    2. Particle diameter (A) = 360
    3. Scale for micrographs = 0.5
    4. Pixel size = 2.08
5. **Particle extraction**
    1. IO, input CTF-job output and manual pick coordinates
    2. Particle box size = 180 (180*2.08A=374A)
    3. Re-scale size = 128
6. **Visualize Extract**
7. **2D Classification**
    1. IO, input extract-job output
    2. Number of classes = 3
    3. Number of iterations = 10
    4. Mask diameter = 360
    5. Use GPU acceleration = yes
    6. Running, --dont_check_norm
8. **Visualize 2D classes**
9. **Continue 2D for 25 iterations**
    1. IO, continue file
    2. Number of iterations = 25
10. **Manual picking, test subset of micrographs for auto-picking**
11. **Subset selection, 2D references**
12. **Auto-picking, test**
    1. IO, input test subset and 2D references
    2. Pixel size in micrographs = 2.08
    3. Mask diameter = 360
    4. Pixel size in references = 2.93 (2.08*180/128)
    5. Shrink factor = 0
    6. Use GPU acceleration = yes
    7. Picking threshold = 1
    8. Minimum inter-particle distance = 300
    9. Running
13. **Visualize, auto-picking**
14. **Auto-picking, real**
15. **Visualize, auto-picking**
16. **Extract**
    1. IO, input CTF-job results and auto-picking coordinates
    2. Re-scaled size = 180
17. **2D Classification**

1. IO, input extract-job results
2. Number of classes = 50
3. Number of pooled particle in RAM = 100
4. Use GPU acceleration = yes
5. Running, --dont_check_norm –maxsig

18. **Visualize, classification**

Part 2

1. **Import particles, star-file**
2. **2D Classification**
    1. IO, input imported particles
    2. Number of classes = 100
3. **Visualize, 2D classes, NOTES: Dead classes**
4. **Subset selection, good classes**
    1. IO, input 2D classification results
    2. Re-center the class averages = no
    3. Regroup the particles = yes
    4. Approximate nr of groups = 100
    5. Select good classes
5. **Particle sorting**
6. **Import reference map**
7. **3D Classification**
    1. IO, input imported particles and imported reference
    2. Ref. Map is on absolute greyscale = yes
    3. Initial low-pass filter = 40
    4. Has reference been CTF-corrected = yes
    5. Number of classes = 3
    6. Mask diameter = 360
8. **Subset selection, good classes**
9. **3D auto-refine**
    1. IO, input imported particles and imported reference
    2. Ref. Map is on absolute greyscale = yes
    3. Initial low-pass filter = 40
    4. Has reference been CTF-corrected = yes
    5. Mask diameter = 360
    6. Number of pooled particles = 100
    7. Use GPU acceleration = yes
    8. Running, --dont_check_norm –maxsig
10. **Inspect map and orientational distribution**
11. **Mask creation**
    1. IO, input 3D refinement map
    2. Initial binarisation threshold = 0.02
12. **Postprocess**
    1. IO, input 3D refinement map and mask
    2. Calibrated pixel size = 2.08
    3. MTF of detector
    4. Number of pooled particles = 100
    5. Use GPU acceleration = yes
    6. Running, --dont_check_norm –maxsig
13. **Visualize, postprocess**