**Smoothing a Mitochondrial Membrane with GAMer 2**

**Objective:** Smooth the meshed contours in Blender for curvature analysis

**Necessary Tools**:

* CellBlender with Gamer 2

**Before Beginning:**

* Follow Protocol 6 to create a .blend file with tiled contours

**Current Smoothing Operation Procedure:**

Outer Membrane

1. Go into edit mode
2. Smooth until significant changes are no longer occurring\*
   1. Max\_Min: 20
   2. Iterations: 10
3. x1 Coarse Dense
   1. CD\_Rate: 2
   2. CD\_Iter: 1
4. x2 Smooth
   1. Max\_Min: 20
   2. Iterations: 10
5. Repeat steps 2-4 as needed.
   1. The final mesh should have approximately [5000] triangles. However, if you need to undershoot this number, you can always complete steps 1-7, use Mesh->Edges->Subdivide, then repeat steps 2-7.
6. x4 Normal Smooth
7. x4 Smooth
   1. Max\_Min: 20
   2. Iterations: 10

Inner Membrane

1. Before performing the boolean operation, repeat the protocol listed above with the IM.
   1. The steps 1-4 must eliminate any major wagon wheels (especially at cristae junctions) before Normal Smooth can be used. Repeat 2-4 if needed.
   2. The final mesh should have approximately [40,000] triangles for a successful boolean operation. However, you will likely need to undershoot this number. 5000 triangles will be enough to achieve a smooth mesh. You can always complete steps 1-4 or 1-6, use Mesh->Edges->Subdivide, then repeat steps 1-6 to achieve something in the 40,000 range.
2. Apply a boolean difference modifier to join the smooth IM and raw CM
   1. Protocol: <https://docs.google.com/document/d/1FR5dIG1bOtZxmMy7ZH7sWQZ5vbyl_pC_EGe24HpVSwk/edit?usp=sharing>

Cristae Membrane + Inner Membrane:

1. Repeat the protocol listed for the outer membrane. Keep in mind:
   1. The steps 1-4 must eliminate any major wagon wheels (especially at cristae junctions) before Normal Smooth can be used. Repeat 2-4 if needed.
   2. The final mesh should have approximately [40,000].

\*One possible error during smoothing is “cannot normalize a vector with zero length”, this could be a degenerated edge in the mesh. To solve this, go to edit mode (vertices), mesh > clean up > degenerate dissolve, and after mesh > faces > triangulate.

**Smoothing a Mitochondrial Membrane with GAMer 1**

**Objective:** Convert IMOD’s .mod file into a Reconstruct series for import into CellBlender.

**Necessary Tools**:

* CellBlender with Gamer 1
  + <http://www.cnl.salk.edu/~bartol/cellblender_bundle/>

**Before Beginning:**

* Follow Protocol 7 to achieve a contiguous inner and cristae membrane structure.

**Current Smoothing Operation Procedure:**

1. Smooth until significant changes are no longer occurring
   1. Max\_Min: 20
   2. Iterations: 10
2. x1 Coarse Dense
   1. CD\_Rate: 2
   2. CD\_Iter: 1
3. x2 Smooth
   1. Max\_Min: 20
   2. Iterations: 10
4. x4 Normal Smooth
5. x4 Smooth
   1. Max\_Min: 20
   2. Iterations: 10