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Supplemental information

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Supporting Material

Building 3-Dimensional Model of Early-Stage Zebrafish Embryo Brain

Ana C. Chang-Gonzalez[1], Holly C. Gibbs[1,2] Arne C. Lekven[3], Alvin T. Yeh[1], and Wonmuk Hwang[1,4,5,6★]

[1]Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA
[2]Microscopy and Imaging Center, Texas A&M University, College Station, TX 77843, USA
[3]Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA
[4]Department of Materials Science & Engineering, Texas A&M University, College Station, TX 77843, USA
[5]Department of Physics & Astronomy, Texas A&M University, College Station, TX 77843, USA
[6]School of Computational Sciences, Korea Institute for Advanced Study, Seoul, Korea 02455
★Correspondence: hwm@tamu.edu

SAMPLE INPUT SCRIPTS

Input scripts below are simplified to illustrate procedures shown in Figure 1. Full scripts are in the `test` folder in our online source code (GitHub: https://github.com/hwm2746/brain-mesh-builder, Zenodo: https://doi.org/10.5281/zenodo.4698489). In the scripts, text after '#' to the end of line is a comment.

Pixel processing

```
#zf_img.inp
# image_list.dat contains input image filenames. The stack is given a tag wt.
img read type stack name image_list.dat tag wt # First read images.
img3d tag wt do
    build img wt0:wt55  # Assign slices 0 to 55 to a stack with tag name wt.
    filter kind gaussian

    # Group slices based on similar intensity profiles (Figure 1A to B).
    filter kind highpass pxl_cut 135 binary img wt0:wt6 # Process slice 0-6.
    filter kind highpass pxl_cut 155 binary img wt7:wt42
    filter kind highpass pxl_cut 158 binary img wt43:wt52
    filter kind highpass pxl_cut 155 binary img wt53:wt55

    # Region extraction and noise removal (Figure 1B to C).
    fill_region pixel_ini 162 64 wsize 2 img wt45:wt46
    fill_region pixel_ini 162 64 wsize 3 img wt47:wt55
    invert
    remove_debris size_cut 20 mode area               # Area-based hole removal.
    remove_debris size_cut 3 mode rank img wt36:wt44  # Rank-based hole removal.
    remove_debris size_cut 4 mode rank img wt45:wt49
    remove_debris size_cut 3 mode rank img wt50:wt55
    invert
    remove_debris size_cut 20 mode area               # Debris removal.

    # Write binary images to ./binary/ folder for input to next step.
    write format tif name binary/wt
    done
STOP
```

BOC and mesh build input

```
#zf_boc.inp
  # Read images and assign to a stack named iwt.
  img read type stack name image_list.dat tag iwt
  img3d build img iwt0:iwt55 tag iwt

  # fnet3d indicates a 3D BOC. Bond length cutoff is 5.
  fnet3d build img3d iwt tag fwt size 5 contour
  fnet3d tag fwt do
      setz dz 1.8750 origin ini       # Slice spacing.
      smoothen_average
      equalize_bond
      clean_filaments                 # Remove filaments containing less than 4 beads.

      assign_bond_z rcut 1.           # rcut is proportional to b0 (size 5).
      prune_bond_z                    # Delete multiple z-bonds.
      fill_enclosed_hole
      assign_bond_z rcut 1.
      prune_bond_z
      smoothen_z delz 2               # Depress protrusions. delz: Propagate to 2 slices.
      assign_bond_z rcut 1.
      prune_bond_z

      clear_bond_z                    # Final refinement.
      smoothen_average
      equalize_bond
      assign_bond_z
      prune_bond_z
      fill_enclosed_hole
      remove_unzbonded_beads

      write type psf+cor name out/fwt # Save data to out/fwt.psf, out/fwt.cor.
      done
STOP
```

SUPPORTING FIGURES

Figure S1: Grid shifting in region extraction. (A) Region extracted with the 3×3 pixel red grid shown in Figure 3B and C. Note the pixelated contour. (B) After merging extracted regions by shifting the grid in 4 diagonal directions. Holes were removed afterwards. This is the image shown in Figure 1C without color inversion.

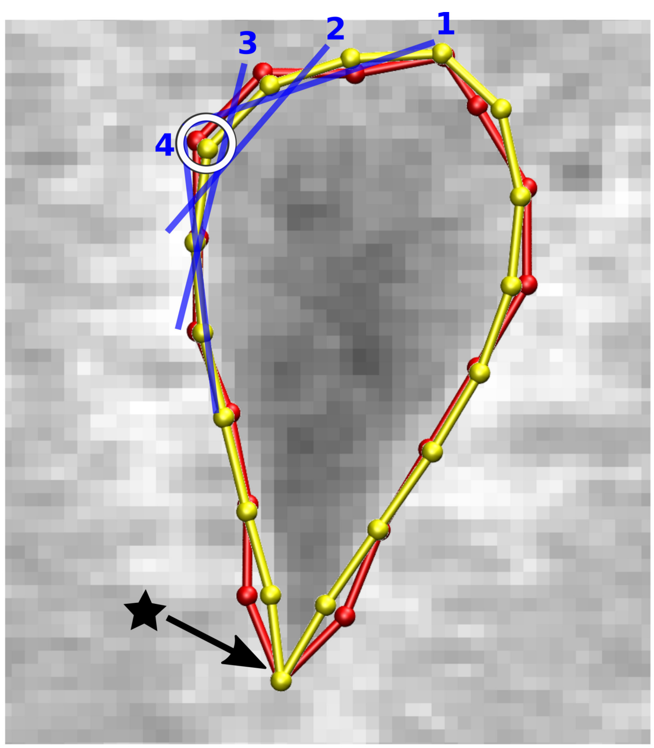

Figure S2: Smoothing BOC. The red BOC is from Figure 4C and the yellow is after 2D smoothing. BOC is overlaid on the corresponding grayscale input image. Blue line is the linear fit of a 4-bead interval. The new position (yellow) of the circled bead is based on the average position from the projections of the original position (red) to the 4 fitting lines. Star points to a sharp bend where the shape of the BOC is maintained with this method.

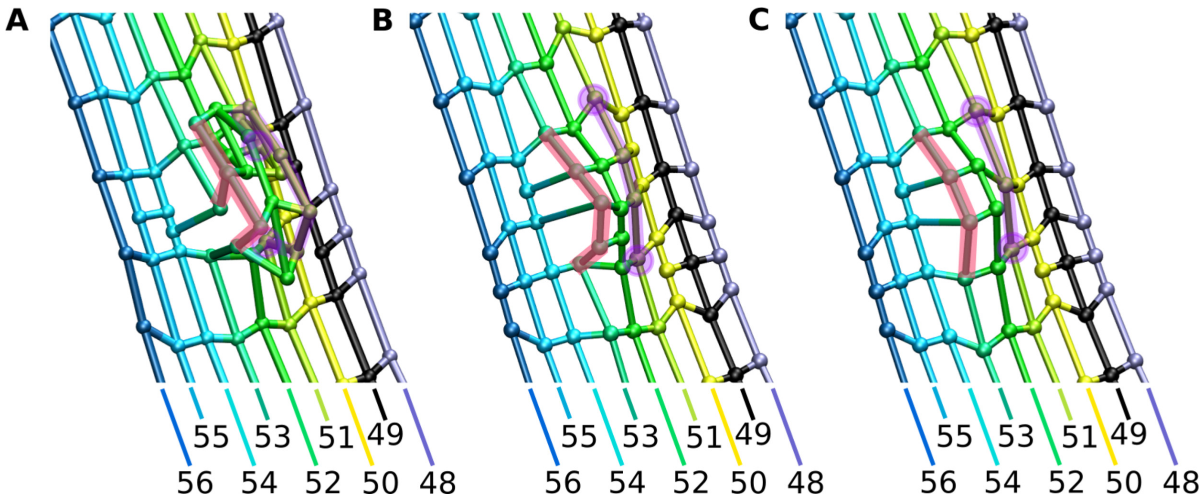

Figure S3: Propagating the effect of depressing a mesh protrusion. (A) Mesh after hole closing step and before moving beads (rotated view of Figure 6E). (B,C) Protrusion in slice 51 (purple highlight) is depressed, which propagates by one slice (B) and two slices (C). In the latter case, slice 53 (red highlight) is also depressed even though its beads are z-bonded (hence not identified as a protrusion), which makes the surface smoother.

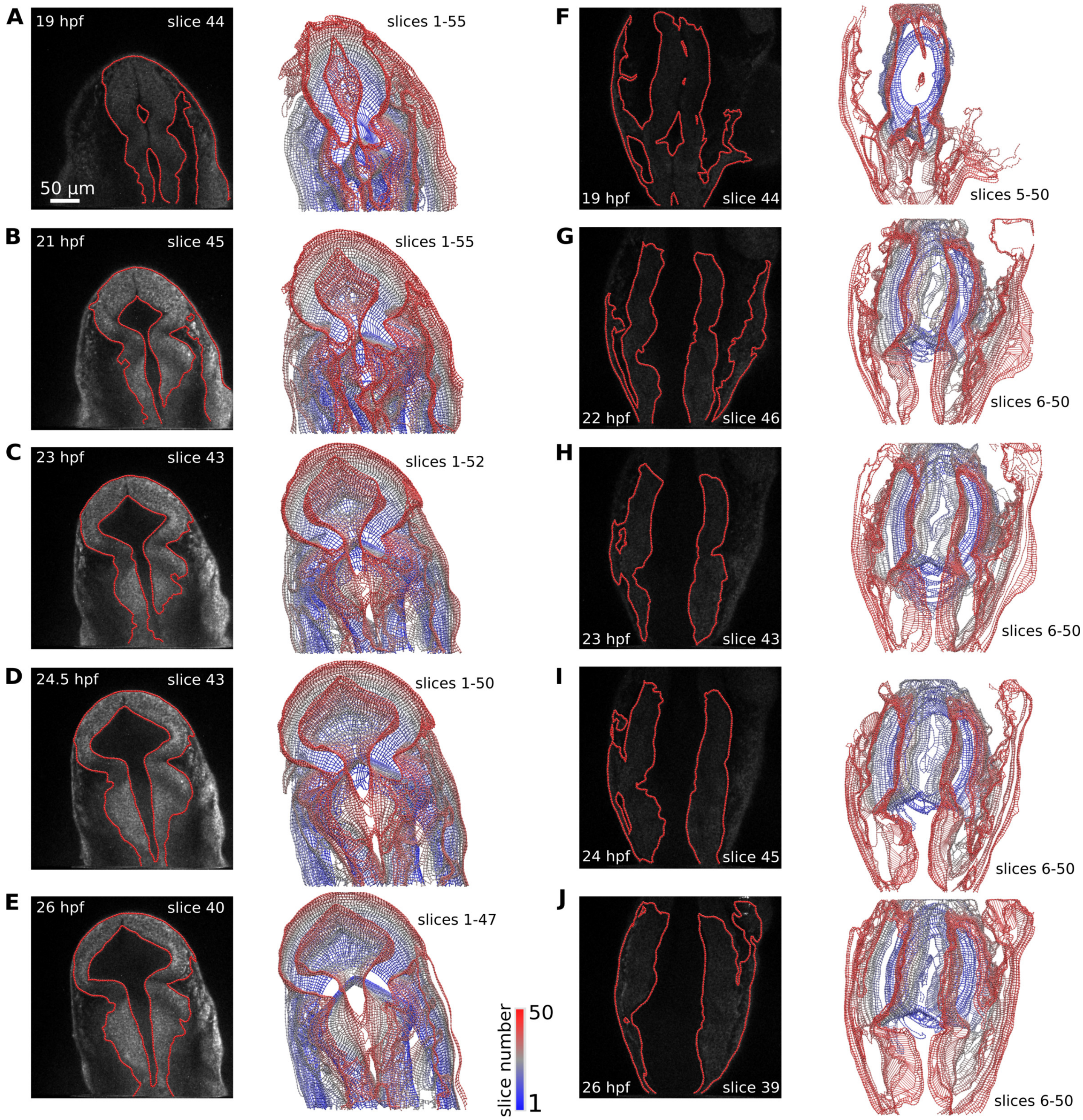

Figure S4: BOC and 3D models at 5 time points for zebrafish live embryos. WT (left column) and *ace* (right column) are shown. These are from the same sets as Figure 7.

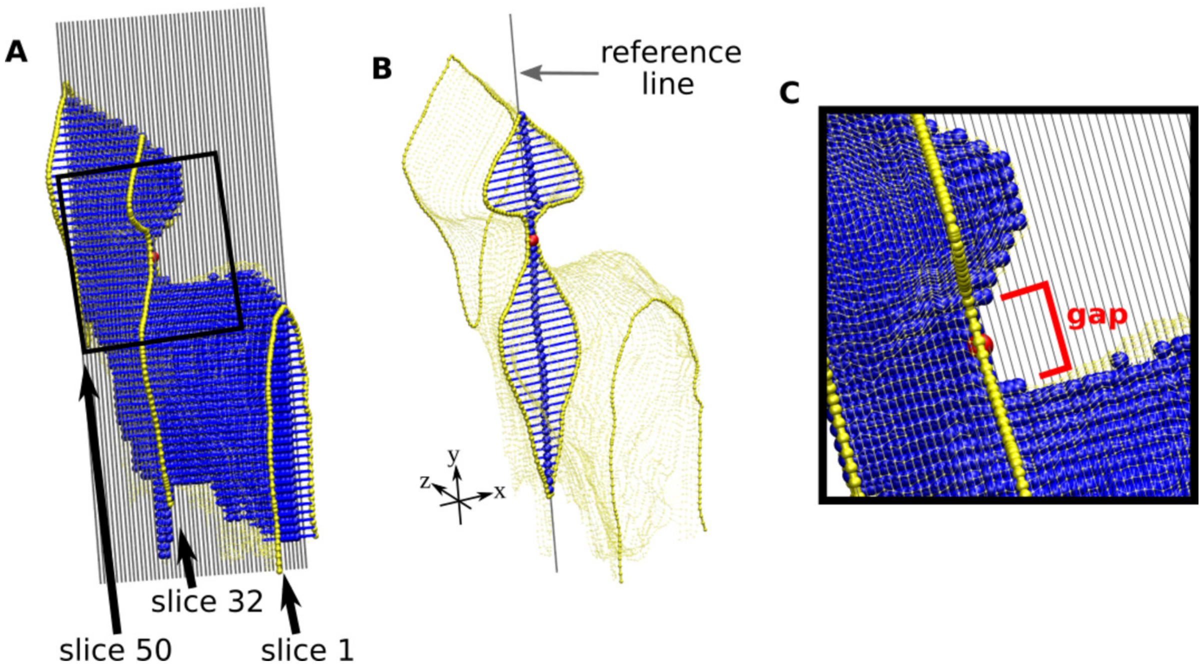

Figure S5: Incremental measurements of the zebrafish embryo ventricle. The WT BOC in Figure 8C is shown. (A) Reference lines span the sagittal plane. 2D BOCs for slices 1, 32, and 50 are shown in yellow. Blue spheres in all three panels represent midpoints. (B) Reference line for slice 32 from the sagittal plane. Blue lines are the incremental ventricle width measurements. (C) Locating the MHB. Magnified view of the rectangle in panel A. Gap in ventricle width measurements before slice 32 is marked, where the first MHB instance is identified (red sphere). It serves as a reference from which the two ventricles and the MHB in the following slices are located.

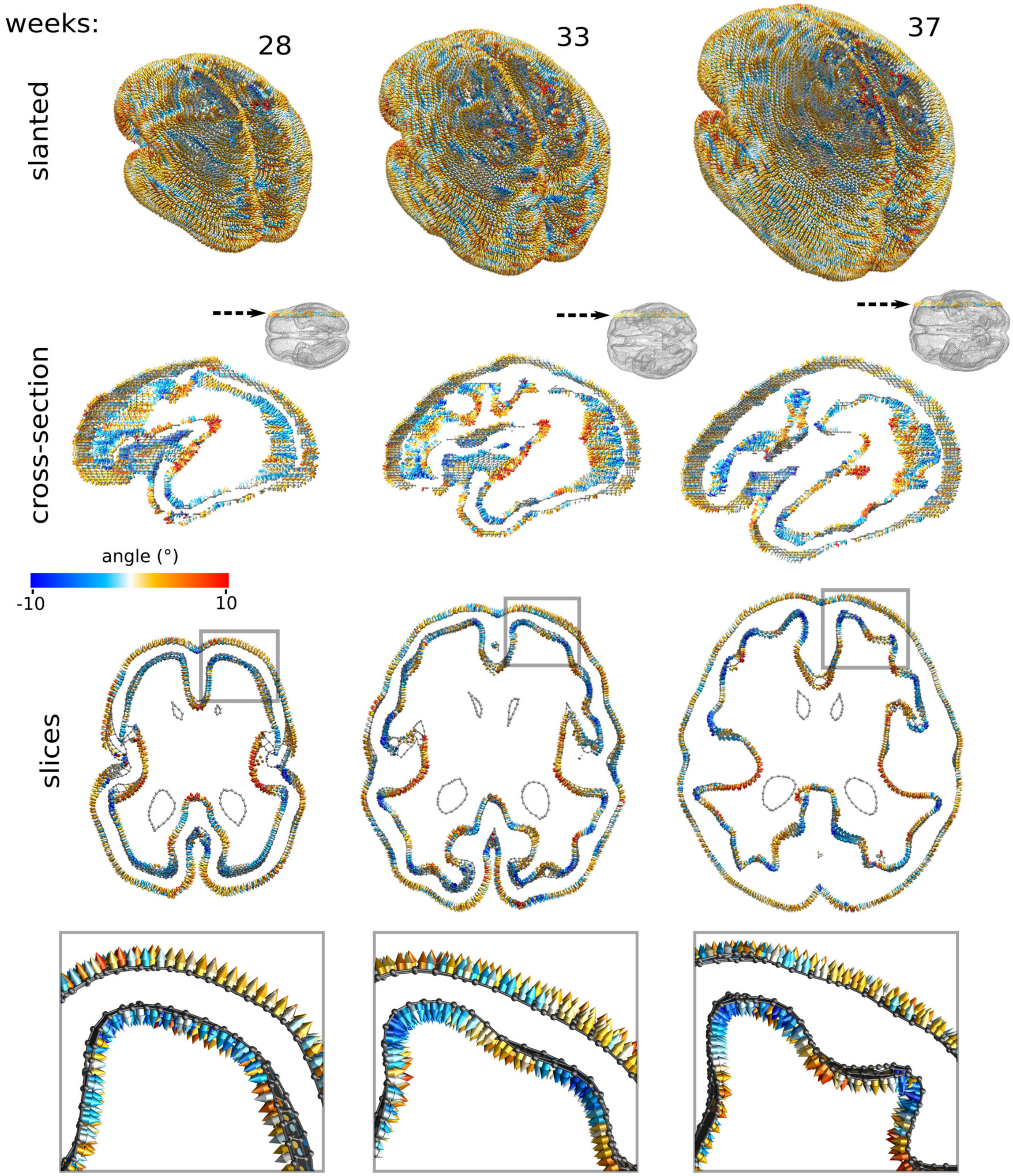

Figure S6: Local mesh curvature measurement from BOC models of the human fetal brain cortex. These are the same models as in Figure 9. Top row: slanted overview. Second row: 6-slice cross-sectional view for the region marked by arrow in the small gray view. Third row: View of the slices in the bottom panel of Figure 9. Fourth row: Magnified views of the rectangles above, revealing outward normal vectors from the cortex. Curvature is measured as the deviation of the average angle between the surface normal and surrounding on-surface vectors from 90° (0° corresponds to a flat surface). Positive and negative angles represent locally convex and concave surfaces, respectively. For presentation, colors saturate for angles beyond ±10°.