

Quantifying Embryonic Torsion

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Introduction: We consider embryonic torsion, which is an important early morphogenetic event that rotates the initially straight body axis. The mechanisms driving torsion are not currently known. Prior studies in our lab and elsewhere indicate that proper progression of torsion is necessary for cardiac looping and brain development. In this study, we used the chick embryo as the experimental model, whose developmental stages have been quantified¹. torsion is a function of position and time, and we quantified it with Optical Coherence Tomography (OCT).

Materials and Methods: Since torsion is position-dependent, three locations were selected: cranial (at the optical cavities level), cardiac (heart level), and caudal sections (at first pair of somites). For each, OCT imaging was used, and different methods were developed to find the corresponding rotation angles in each of the relevant HH stages: 12 (~48 hrs. of incubation) to 14 (~56 hrs.). The embryo's cross-section images were obtained using the OCT data acquisition system and processed in MATLAB. For each section, angles were obtained using the petri dish surface line as a reference (embryos were cultures *in vitro*). The procedures employed for each location were:

Cranial: The center of each optical cavity was used as a reference point. As Fig. 1B&C show, an angle of rotation was obtained using the coordinates of each point. The angle of rotation of the optical cavities was obtained by comparing the elevation between the two cavities and the petri dish surface.

Cardiac: At the location of the maximum bulge of the bulbo-ventricular loop, the centers of the neural tube and notochord were used as reference points. This is the green line that crosses the heart (HR) in Fig. 1A. The neural tube was used as a rotational point with respect to the notochord. To normalize these values, the angles obtained were subtracted from 90°, as the unrotated section starts at approximately 90° and decreases over time.

Caudal: A line joining the center points of the first pair of somites underneath the anterior intestinal portal (AIP) was used as a reference.

Results and Discussion: The results show that, between stages HH 12 and HH 14, the cranial section (n = 11 for HH 12, n = 9 for HH 13, n = 5 for HH 14, respectively) rotates approximately 72°, the cardiac section (n = 10, 8, & 5 respectively) about 45°, and the caudal section's (n = 10, 6, & 3 respectively) rotation is negligible. This confirms prior qualitative analyses, which indicate that rotation starts cranially and progresses caudally in these stages. As expected, in these stages, rotation in the cranial section is nearly complete, while rotation in the caudal section has not yet started.

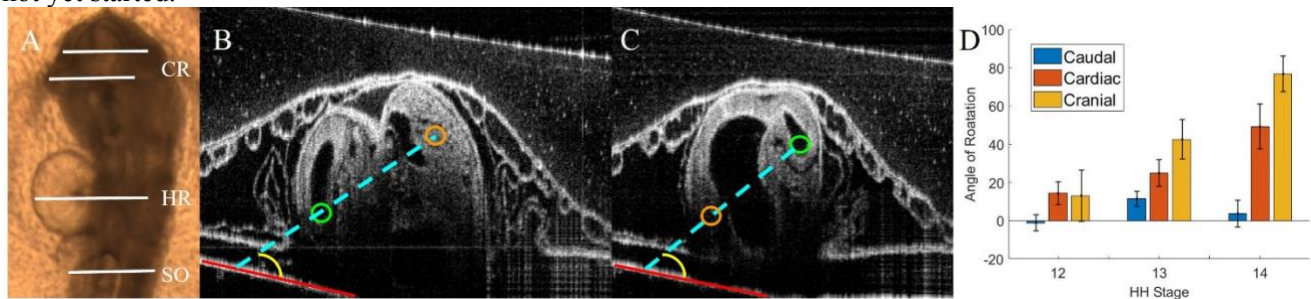


Figure 1. Embryonic rotation at HH 13 under control conditions. OCT images of cranial cross sections are also shown. **A:** Embryo at HH 13. White lines represent the cross sections taken at cranial (CR), cardiac (HR), and caudal (SO) sections. **B&C:** Method used for quantification in cranial region. Cross sections where either the top optical cavity is visible (Fig. 1B), matching the top CR line in Fig. 1A, or the bottom optical cavity is visible (Fig. 1C), matching the bottom CR line in Fig. 1B. Solid green circle encloses the visible optical cavity in the cross section. Orange circle encloses the optical cavity that is not visible in the cross section. Red line is used for reference, as it runs across the rigid petri dish surface. Light blue dashed line represents the distance vector between the two optical cavities. Yellow arc represents the angle of rotation of the optical cavities, with respect to the petri dish surface. **D:** Graph representing the angles of rotation of caudal, cardiac, and cranial sections at HH stages 12, 13, and 14. Black error bars represent standard deviations.

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References: ¹Hamburger V and Hamilton HL, J Morphol, 1951, 88, 56