

Post-embryonic imaging of zebrafish semicircular canal morphogenesis

The vertebrate inner ear contains three orthogonally arranged semicircular canals that function to detect angular accelerations (turning movements of the head). Each canal comprises a curved duct with a swelling (ampulla) at the base that houses sensory hair cells. We are using light-sheet fluorescence imaging of transgenic zebrafish to examine formation of the semicircular canals from embryonic to adult stages. This technique is particularly well suited for this purpose as it allows cellular details to be resolved in relatively deep-lying tissue ($>100\ \mu\text{m}$) within a large whole-mount specimen (1-10 mm). We are using 3D renderings of our data to visualise the changes that occur during the post-embryonic period. Additionally, we are making morphometric measurements of the tissue, including canal duct length, lumen diameter and angle between the canal ducts. We are using these measurements to generate an atlas of wild-type semicircular canal development as a reference point for comparison with mutant phenotypes.

Our imaging is revealing detail that has not previously been observed using paint-fill or histological techniques, to our knowledge. All three canal ducts have a distinct morphology from the time of their appearance at 3 days post fertilisation. The anterior and posterior semicircular canals form as ducts first and enlargement to form their ampullae happens at later stages. Conversely, in the lateral (horizontal) canal, the presumptive ampulla is already evident as a swelling around the lateral crista at 3dpf, and the lateral canal duct elongates during post-embryonic stages. These differences between the lateral canal and the other two canals correlate with different genetic requirements: for example, the lateral canal is uniquely dependent on the conserved function of *otx1b*. We have also observed other anatomical features including a notable seam along the length of each canal and a thickening of the epithelium at the sides of the ampulla in adult inner ears.

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