University of Cambridge
Faculty of Mathematics
Summer Research Projects

Symmetry-breaking in Zebrafish development

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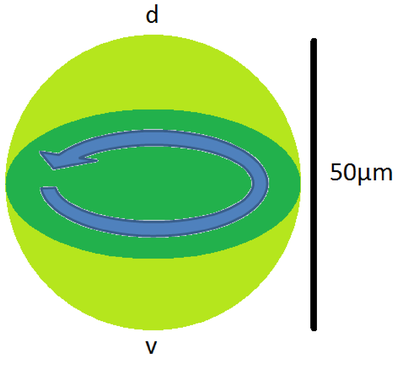
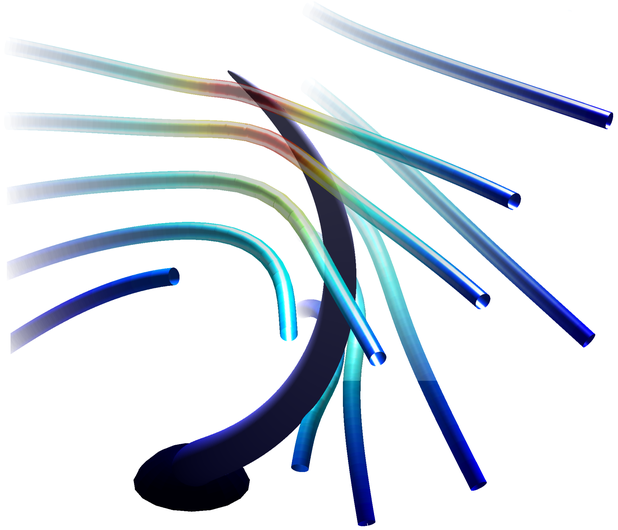
Symmetry breaking in animals

Initially, many animals – including mice, zebrafish and humans – appear bilaterally symmetric. Whilst front and back, top and bottom are clearly defined, there is little difference between the left- and right-hand sides. On closer inspection, however, we see that organs are arranged systematically in a non-symmetric way, for example the heart lies primarily on the left-hand side. The development of these axes early in development is an interesting question of Fluid Dynamics. The left-right axes are the last to develop, their development depending on a directional fluid flow in some organising structure.

In zebrafish (right), a tiny fish commonly looked at by developmental biologists, the structure controlling this symmetry breaking is called **Kupffers Vesicle** (KV), transient, spherical, fluid-filled organ. In zebrafish, KV is generated from a cluster of 20-30 dorsal forerunner cells which combine to create a ciliated epithelium. These cilia are motile and drive an anticlockwise fluid-flow in the plane normal to the dorsal-ventral axes.

Our question is how we model these cilia – tiny hair like structures on the surface of the cells – and further how we can model the fluid flow induced in three dimensions. Computationally, full modelling is difficult, so a variety of simplified models were compared to deduce whether computational efficiency could be obtained without sacrificing quantitative accuracy. Comparing the flow induced by the model of cilia within a spherical geometry with the known experimental result allows us to deduce results about the direction of cilia tilt, and explore the flow inside KV away from the (experimentally studied) mid-plane, illustrated below.





Mathematical Modelling

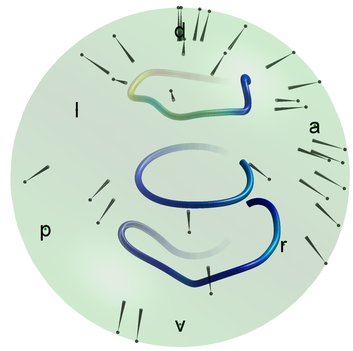
The full equations governing fluid flow around cilia are known. Due to the small size of the relevant geometries and speeds, the flow is described by the **Stokes equations**, the zero-Reynolds number limit of the Navier-Stokes equations. The fundamental solution to these equations (in all of space) is known, and called the Stokeslet. Differentiating this solution leads to other fundamental solutions, which can be combined to give solutions in more complicated geometries, for example the flow generated by a point force near an infinite sheet, on which the flow vanishes.

Unfortunately, for even moderately complicated geometries solutions to the Stokes equations are not known. However, powerful numerical techniques have been constructed which can cope with these. Initially, we modelled a cilium by a string of 'regularised' Stokeslets: instead of a line of point forces (a multiple of a δ-"function"), the forces were slightly de-localised (instead being a multiple of a local but finite radial "blob" function which integrated to 1, tending towards the δ-function as the localisation parameter tended to 0). By fixing the motion of the cilium (rotating about a tilted axis) and insisting the generated fluid flow matched the cilium velocity at any point along it, we could in turn compute the flow field throughout the domain.

This is fine for infinite and half-infinite domains, where analytic expressions for the singularities are known. However, it is a numerically taxing routine. We deduced that when time-averaged, the fluid flow not far above the cilium corresponds to that of a point torque. By applying the **Boundary Integral Method** for Stokes flow – essentially replacing integration within some domain with integration on its boundary – we can utilise this point torque model in more complicated domains, in our case a simple sphere. Having successfully reduced the complicated, fully-resolved model of a cilium to a single point singularity, we can efficiently compute the (three-dimensional) flow in KV.

Results

There is some disagreement in the biological community about which way the cilia in KV tilt – do they tilt towards the rear (posterior side, p) or top roof (dorsal side, d) of KV? Further, the cilia are not evenly distributed around the cell. Taking these various possibly tilts into account allowed us to construct a variety of different flows in KV. Shown below are some streamlines induced by dorsal tilted cilia at the positions shown by the black shapes – it corresponding in magnitude and qualitative behaviour very closely to that experimentally observed.



Further, this model allows us to observe the individual contribution from each cilium. Careful analysis revealed that almost all the cilia, those on the ventral roof (the bottom of KV, v) aside, contributed to the experimentally-observed anticlockwise flow. Indeed, one can see from this individual flow that the distribution of cilia is such as to maximise this anticlockwise flow, which holds if we have dorsal tilt. Thus, we determined two things – firstly that the cilia are most likely tilted dorsally, and secondly "why" the non-uniform distribution of cilia is as it is.

Potential Further Research

It was noted that for a curved cilium, there was an additional "corkscrew"-like effect, in which the fluid would be expelled from the cilium (in a time-averaged sense). This can be modelled by a combination of Stokeslet (point-force) and rotlet (point-torque) singularities. What is the effect on fluid flow in KV?