

Figure 1. Mounting system for an early zebrafish embryo (30 hpf). a) Mold which has a shape similar to the zebrafish (zebrafish embryo shown for comparison). The mold is then placed into a petri dish filled with 2% liquid agarose. After the agarose has solidified, the mold is removed and the zebrafish embryo is placed into the created ridge. Finally a small layer of 1% agarose is placed on the tail of the embryo to prevent movement of the embryo. b) Final configuration of the mounting system. The pectoral fins are not covered by agarose, only by medium and are free to grow and develop. Since the tail of the embryo is fixed, the microscope has to be repositioned every 8 hours to account for the change in length of the embryo. With this system, time-lapse movies of up to 32 hours can be made.

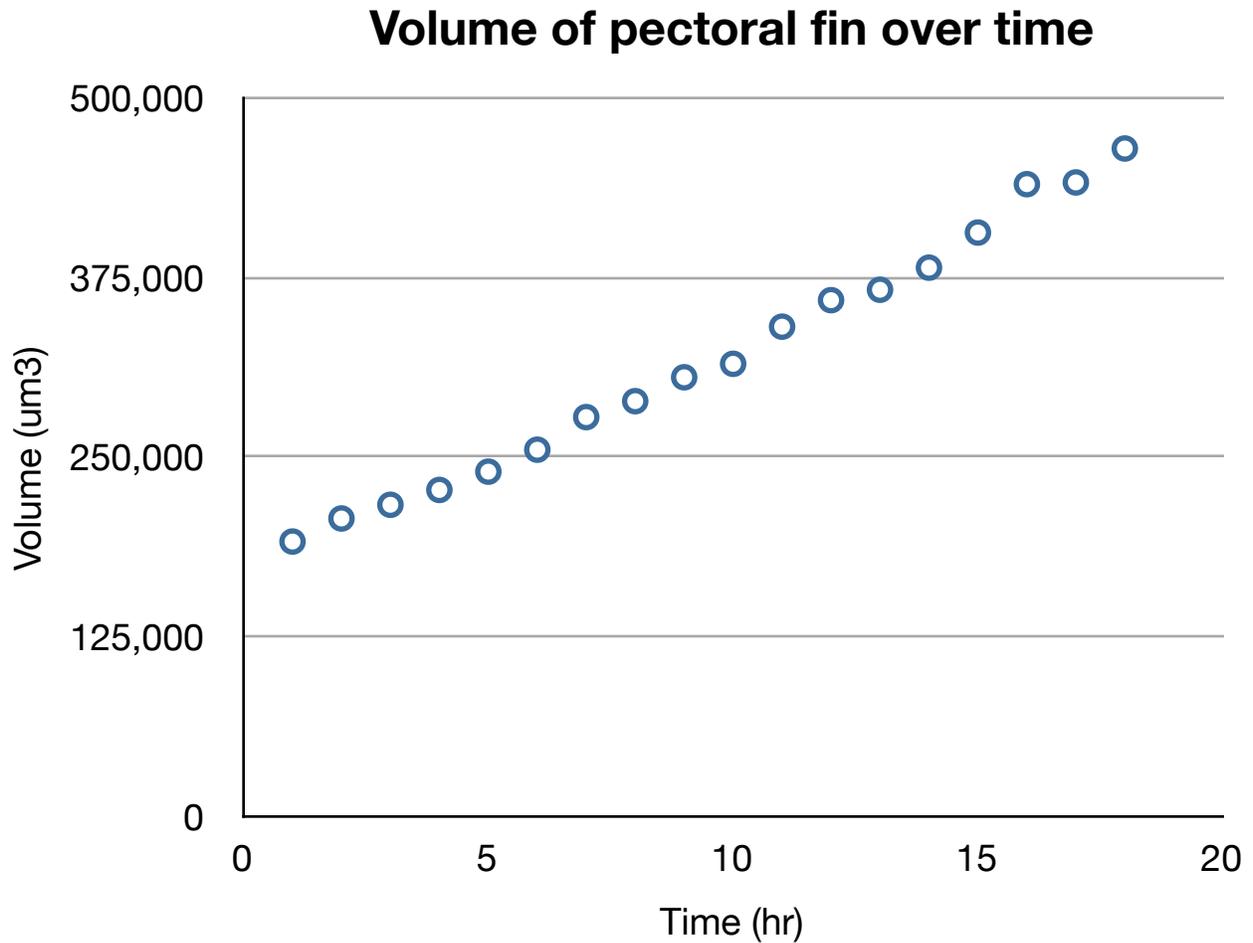


Figure 2. Volume of the pectoral fin during early embryonic development. $t=0$ hr equals 30 hpf. The fin doubles in size every 12 hours. The cell cycle length was estimated to be 25 hours, determined from Phospho-Histone 3 antibody stainings. Therefore cell division by itself is not sufficient to account for the growth of the pectoral fin and other mechanisms (i.e. inflow of cells from neighbouring tissue or changes in the volume of individual cells) have to play a role.

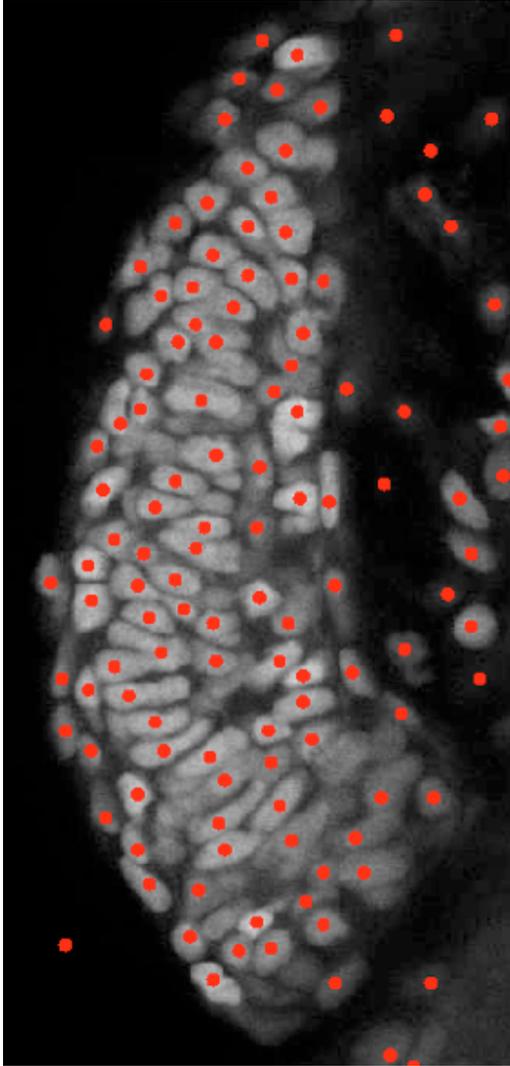


Figure 3. Result of the cell nucleus detection algorithm. One plane of a z-stack of a pectoral fin of a 48 hpf H2A-GFP transgenic zebrafish embryo is shown. Red dots indicate the cell nuclei detected by the algorithm. The algorithm has a success rate of > 90%.

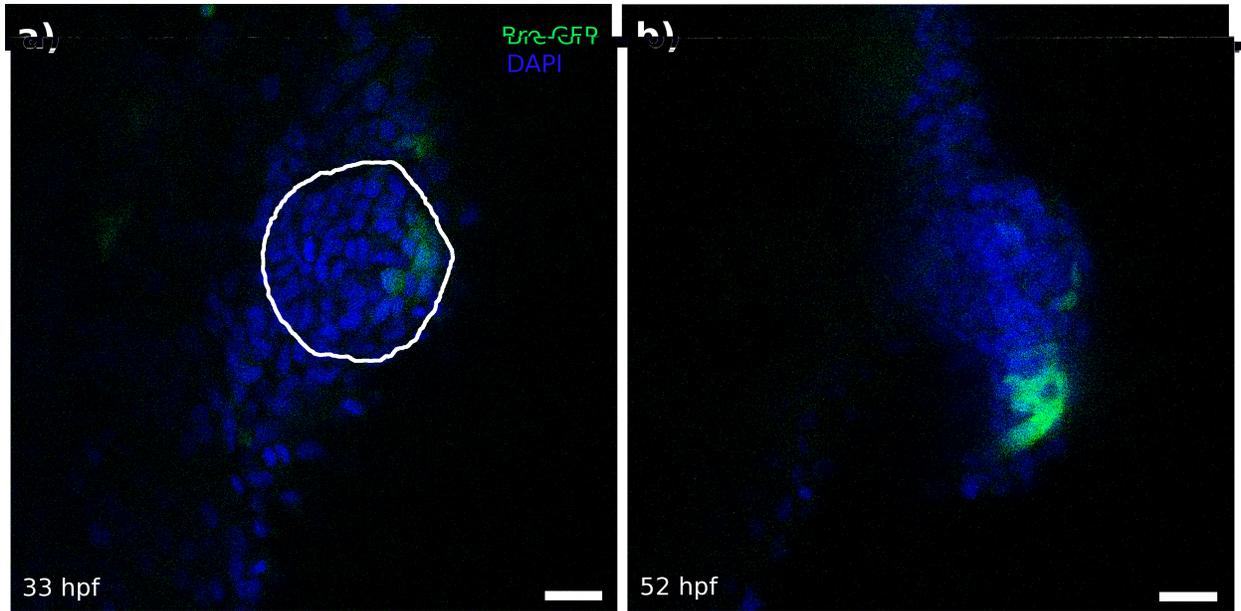


Figure 4. Bre-GFP expression in the zebrafish pectoral fin (right fin) at 33 hpf (a) and 52 hpf (b). One slice of an image stack is shown. DAPI staining (blue) is used to show the cells of the fin, Bre-GFP is shown in green. The white line in (a) indicates cells which are part of the pectoral fin bud. In (b) all visible cells are part of the pectoral fin. In both images Bre-GFP expression is clearly visible. In 52 hpf embryos expression, and hence Bmp signalling, is stronger than in 33 hpf. Anterior is up, posterior is down. Scale bar = 20 μ m.

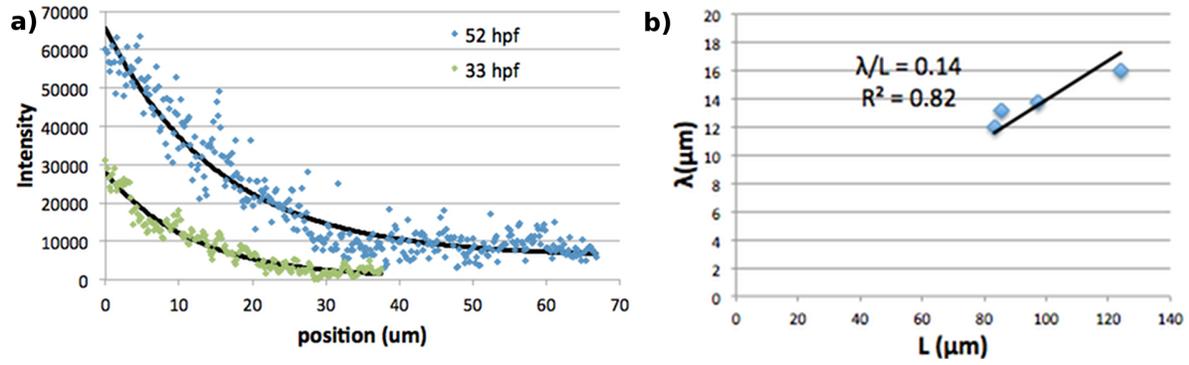


Figure 5. Preliminary results of Bmp signalling in the zebrafish pectoral fin. a) Gradient of Bmp signalling observed in the zebrafish pectoral fin. At 33 hpf the gradient has a decay length of 12 μm , while at 52 hpf this is increased to 16 μm . b) The decay length λ scales with the length of the pectoral fin.