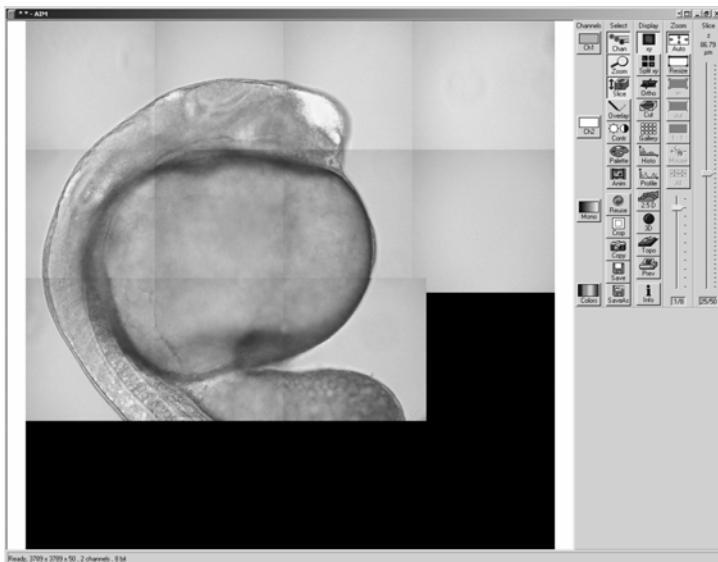


# Confocal imaging of large areas of biological objects at high resolution

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Imaging of large areas of thicker biological objects such as a 48 hpf (hours post fertilization) zebrafish embryo at high resolution in 2- to 5D with confocal microscopy is, even after the fast development of microscope technology of the last years, still limited by technical obstacles. Our aim is to image a field of  $x-y > 300 - 5000 \mu\text{m}$  and  $z > 200 - 500 \mu\text{m}$  with a pixel size of  $0.2 - 0.5 \mu\text{m}$  resulting in images of approximately  $10000 \times 2000$  pixel and 400-1000 layers. In a first step, a Visual Basic macro was programmed allowing for acquisition of multiple  $x-y$  overlapping image sequences in 3D. The macro also included an algorithm, which calculated out of the multiple stacks a large single final image stack after the acquisition. Several problems occurred with this procedure. The successful matching of the overlapping images was strongly dependent on the image quality as well as structures in the image. In addition the precision of the motorized table and flatness of the sample holder on the microscope was not sufficient to always achieve good matches of the images. In thick specimens the signal strength in deeper layers was too weak even when using 2-photon excitation. Therefore, we developed a special closed mounting chamber for zebrafish embryos, which allows sequential imaging of the embryo from both sides. The chamber is designed to be completely symmetrically. It can be turned around after taking  $z$ -stacks from one half or  $2/3$  of the embryo to image the other side of the embryo thus avoiding the poor image quality in the very deep layers. The resulting image series – up to 10 GB - have then to be matched in  $z$  as well. This is work currently in progress together with the Institute of Computer Science (University of Freiburg, Department of Pattern Recognition and Image Processing, Prof. Burkhardt).



During the acquisition the full area to be scanned is displayed tile after tile. The tiles positions are correlated to each other with the parameters given by the user in the macro. It is possible to view during the ongoing tile scan, the acquired images in  $z$ . The images can be later stored as a correlated full size image stack or as a multiple stacks of tiles to later optimize the correlation parameters if necessary.