In vivo high field magnetic resonance imaging and spectroscopy of adult zebrafish

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Summary

The zebrafish model combines the relevance of a vertebrate with the scalability of an invertebrate and, in some studies, could provide an interesting intermediate vertebrate model to laboratory small mammals. In addition, the zebrafish genome is available as a preliminary assembly, and numerous mutant lines have been generated. It is clear from the genome studies that practically all disease genes in humans have counterparts in the zebrafish. Among various diseases, zebrafish has also recently entered the stage as a promising model system to study human cancer and neurodegenerative diseases. A better understanding of the comparative anatomy and physiology of adult zebrafish will be required, and *in vivo* imaging methods will be very important to bridge this gap. MRI is an imaging technique that can provide access to adult zebrafish anatomy with good resolution. It has been applied at embryonic stages, but not yet in the adult fish. MRI in conjuction with MRS can be in valuable for studying disease at molecular levels.

This thesis contains the results of imaging of adult zebrafish by using different MR approaches. The relevance of this work is summarized in **Chapter 1**, in addition to a brief introduction of MRI and MRS methods and their relevance to study adult zebrafish.

In C**hapter 2**, we present the first high resolution μMR images of adult zebrafish. To achieve high resolution we used a magnetic field of 9.4T, in

combination with strong magnetic field gradients (1000 mT/m) and specialized radio frequency coils were used to achieve high spatial resolution. To support imaging of living fish, we designed a special flow-through setup for continuous flow of aerated water to support living zebrafish inside the magnet. Clear morphological proton images were obtained by T_2-weighted RARE sequences revealing many anatomical details in the entire intact zebrafish *ex vivo* as well as *in vivo*. In addition, a 3D model of zebrafish was annotated from µMRI image slices which allowed complete three-dimensional models of various structures such as brain, heart, liver, and swim bladder are constructed.

Zebrafish is increasingly used as a model organism for understanding brain diseases; however, there is an apparent lack of information about the metabolic composition of zebrafish brain. It is well known that the brain metabolites are sensitive indicator of various pathological processes. The *in vivo* assessment of brain metabolites and tracking the changes in metabolic profile over time will be indispensable tools to understand disease progression and its mechanism. MR spectroscopy is a non-invasive tool that can be used to measure the chemical composition of zebrafish brain *in vivo*. In **Chapter 3**, we successfully implemented MRS at 9.4T and obtained for the first time detailed composition of zebrafish brain *ex-vivo* as well as *in vivo*. Our results in this chapter suggest that zebrafish brain has similar metabolite profile as the human brain, which proves that zebrafish is a good model organism to study human brain disorders.

Zebrafish models have created their own niche in cancer research. Most of the tumors in zebrafish develop late in life, and the non-invasive imaging tools to detect tumor development in adult zebrafish are lacking. **Chapter 4** demonstrates the application of high resolution μMRI methods, which were developed in chapter 2, to track spontaneous tumors in stable transgenic zebrafish models expressing a RAS oncoprotein and lacking P53 (mitf:Ras::mitf:GFP X p53-/-). Tumors were successfully visualized at different locations in live zebrafish. In addition to imaging melanomas in zebrafish at moderately high field (9.4T), we applied ultra-high field (17.6T) to obtain better sensitivity and resolution. Imaging at ultra high field revealed significant tumor heterogeneity which was confirmed by considerable changes in transverse relaxation time, T_2 measured in various regions of tumor. These results demonstrated the feasibility of μMRI technique to non-invasively monitor tumors and their anatomy in living adult zebrafish. Such non-invasive μMRI may allow longitudinal studies of tumor development and real-time assessment of therapeutic effects in zebrafish tumor models.

At the end, **chapter 5** provides a general discussion of the work presented in this thesis and a future outlook.