Imaging the whole life span of a Zebrafish with Magnetic Resonance Microscopy (MRM) using a standard bore 850 MHz system

Thomas Neuberger[1,2], Tatjana Neuberger[1], Darin Clark[3], Victoria Braithwaite[4,5], and Keith Cheng[3]

[1]Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, United States, [2]Department of Bioengineering, Pennsylvania State University, University Park, PA, United States, [3]Department of Pathology, Penn State College of Medicine, Hershey, PA, United States, [4]School of Forest Resources, Pennsylvania State University, University Park, PA, United States, [5]Department of Biology, Pennsylvania State University, Univerity Park, PA, United States

Introduction:

The zebrafish is now widely recognized as a powerful vertebrate model system. Its experimental features, which include transparency, fecundity, and strong genomic tools, have made it an attractive, tractable model in basic biology through to more complex studies of disease. During its early transparent developmental stages light microscopy is an excellent tool for imaging the animal. As the fish continues to develop, however, increasing pigmentation demands alternative imaging modalities for assessing morphological changes. With its ability to create three dimensional images of nontransparent specimens, noninvasive MRI is a promising imaging modality that can be applied. Recently, Ullman et al. [1] published a detailed three-dimensional atlas of the adult zebra fish brain ex vivo. Kabli et al. [2,3,4] have also shown initial results acquired from living specimens; they described the morphology [2], an application on malignant melanoma [3] and, by using magnetic resonance spectroscopy, the metabolite profile of the adult zebra fish brain. Here, we used high resolution magnetic resonance microscopy (MRM) to image zebrafish embryos through different developmental stages from day one post fertilization (1dpf) through to the adult stage (2 years).

Subjects & Methods:

Fish of different age groups were euthanized with Finquel (Sigma, USA) and fixed with a 10% neutral buffered formalin solution (VWR, USA). After fixation the specimens were immersed into a 2% Magnevist (Bayer, Germany) solution for 1 week to reduce the imaging time. All experiments were conducted on a 20 tesla ultra shielded Bruker Avance III 850 (Bruker Biospin, Billerica, MA, USA) standard bore vertical sytem. Three dimensional gradient echo images (TE = 4 ms; TR = 25 ms) were acquired using a commercial 5 mm ID saddle coil for the smaller animals, and a 10 mm ID saddle coil for the larger animals, respectively. Imaging time varied from 5 – 18.5 hours depending on the zebrafish size and resolution. The resolution was 20 μm isotropic for the larger animals and up to 8 μm isotropic for smaller specimens. During reconstruction (Matlab; The Mathworks, Inc., Natick, MA) zerofilling by a factor of two in each direction resulted in a pixel resolution of up to 4 μm isotropic. Image segmentation and volume measurements were performed using AVIZO (VSG3D, USA).

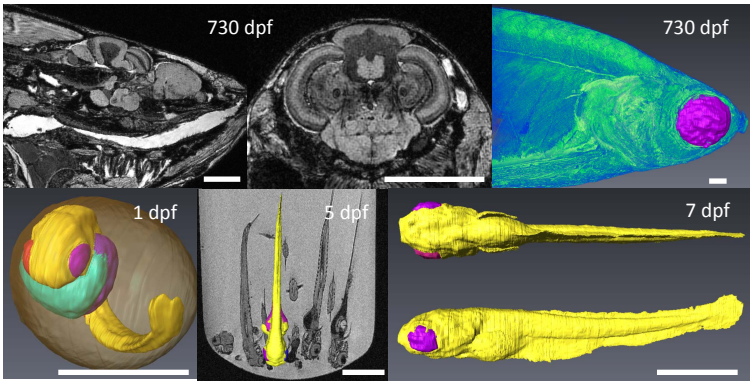

Figure 1: MRI images and reconstruction of the developing zebrafish. The images on top show the brain of a 2 year old fish, those underneath are reconstructions of fish from very early developmental stages (1,5, and 7 dpf). The white bar in the panels represents the length of 1 mm.

Results:

The upper row of Figure 1 shows the brain of the 2 year old specimen. Owing to the high contrast to noise ratio we achieved a higher level of detail compared to [1] as well as to a histology based zebrafish brain atlas [5] (data not shown). As the zebrafish in their early developmental stage are small compared to the RF resonators that were used, up to 60 specimens could be scanned at the same time increasing the throughput and therefore reducing the imaging time per specimen to less than 20 minutes (in detail: 1dpf/2dpf: more than 60 specimen/scan; 4dpf/5dpf: more than 30 specimen/scan; and 7dpf: 12 specimen/scan). The lower row of Figure 1 illustrates reconstructed zebrafish at 1, 5, and 7 days post fertilization (dpf). At 1 dpf the fish is still inside the egg. The reconstruction shows that besides the yolk sac and the body the eyes can be identified. Early developmental stages of the fins can be seen at 5 and 7dpf. During the development (1dpf – 2 years) the eye grew from 0.002 mm^3 to 4.8 mm^3 (data not shown). Th e length of the brain increased from .5 mm at 4dpf to 4.5 mm at two years, while its volume increased from 0.02 mm^3 (4dpf) to 6.4 mm^3 (2 years) as illustrated in Figure 2.

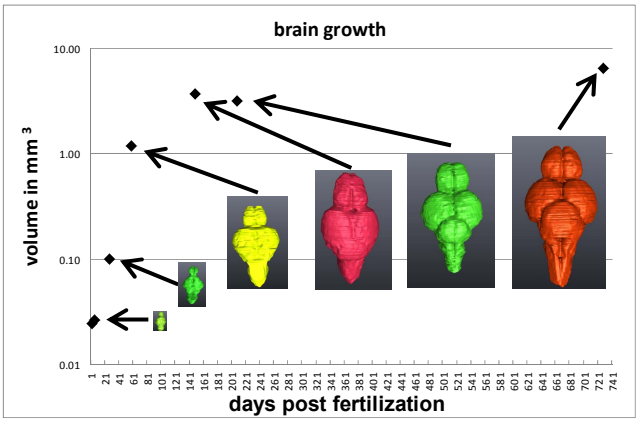

Figure 2: Volume change of the developing zebrafish brain. Segmentation started at 4dpf with a volume of 0.024 ± 0.002 mm^3. The volume of the 2 year old specimen was 6.45 mm^3.

Discussion:

This study demonstrates that MRI provides an excellent tool with which to image the full life-span of the zebrafish at a high resolution. The high magnetic field of 20 tesla and the extremely high magnetic field gradients (3000 mT/m) allowed a spatial resolution of up to 8 microns isotropic not achievable at low magnetic field strengths. Furthermore, due to the small size of the specimen in their early developmental stages, a high throughput of more than 60 zebrafish embryos per scan was achieved. The data we present provides information on the development of the zebrafish that is similar to work conducted by other groups [1, 2], but we have been able to resolve finer details across different stages of development. Our research is now focusing on the development of smaller, more sensitive resonators that will achieve an even higher resolution and moving towards *in vivo* imaging that will permit us to study the progression of disease stages, such as those associated with melanoma.

References:

[1] Ullmann et al, Neuroimage, 51:76-82, (2010). [2] Kabli et al, Zebrafish, 3(4):431-9, (2006). [3] Kabli et al, Zebrafish, 7(2):143-8, (2010). [4] Kabli et al, JMRI, 29:275-281,(2009). [5] Wulliman et al, 'Neuroanatomy of the Zebrafish Brain: A Topological Atlas', Birkhaeuser, 1995

Acknowledgements:

Supported by NIH grant 2R24RR017441 from NCRR to KC. We like to thank the NMR facility at the PSU Chemistry Dpt. for their great support.