In vivo MR imaging of zebrafish with focus on cardiac tissue

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Target audience – Scientists and clinicians interested in zebrafish MRI.

Purpose

Zebrafish are valuable models to study cardiac regeneration [1]. In the evaluation of the effects of interventions it is important that heart function can be assessed by live imaging, but this is challenging in the adult zebrafish [2]. An attractive option for non-invasive visualization is *in vivo* high resolution MRI. *In vivo* MRI of zebrafish has been performed [3,4], but motion artifacts due to movement of the water surrounding the zebrafish and movement of the fish itself hampers proper cardiac imaging. Furthermore, the in-plane resolution for heart visualization is currently suboptimal. More recently, a study was presented in which a retrospective self-gated cardiac MR sequence for Cine cardiac MRI was applied [5]. However, this was done *ex vivo* in the post mortem period that the heart was still beating and also spatial resolution was limited.

The *aim of this study* was to develop a setup for μMRI of live zebrafish with limited motion artifacts and an in-plane resolution down to 25x25 μm. We also present *in vivo* cardiac images of the zebrafish heart using the IntraGate brightblood Cine FLASH sequence.

Methods

Zebrafish were anesthetized by immersion in a mixture of 0.032% (wt/vol) tricaine in water until loss of righting reflex and of response to external stimuli. The fish was then wrapped into a thin layer of gauze bandage soaked in a 0.008% (wt/vol) tricaine mixture and then positioned in a customized 2,0 ml Eppendorf with tubes at both ends filled with a 0.008% (wt/vol) tricaine mixture. The Eppendorf tubes were connected to a syringe filled with a 0.008% (wt/vol) tricaine mixture, enabling a manual flush to refresh the complete amount of water inside the tube (figure 1). In this way the anaesthetized zebrafish is in the same water for a maximum of 15 minutes. The fish survived for at least 35 minutes in the MRI and showed complete recovery.

MR measurements were performed on an 11.7 T BioSpec Avance III animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with actively shielded gradients of 600 mT/m.

A homebuilt Tx/Rx coil was designed consisting of a 2 turn balanced solenoid coil (length 10 mm and inner diameter 11 mm), fitting tightly around the Eppendorf tube containing the zebrafish. Mechanics were added to position the coil horizontally perpendicular to the B0 field (figure 1). The coil was tunable to 500,4 MHz and Q factors of the coil are $Q_{unloaded}$=190 and Q_{loaded}=80.

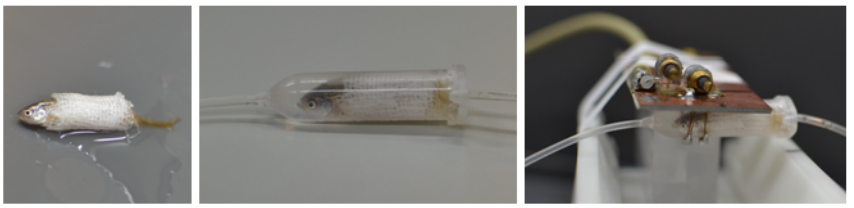

Figure 1: Photos showing the positioning of the zebrafish in the MRI setup, with (a) the zebrafish wrapped in gauze bandage, (b) the zebrafish in the customized Eppendorf and (c) the Eppendorf inside the MRI coil.

The MRI protocol started with a fast TriPilot scan for localization followed by a FLASH sequence of 9 slices (Tr=800 ms, Te=6.28 ms, Flip Angle (FA)=40°, slice thickness (ST)=0.25 mm, FOV=25.6×25.6 mm, matrix size=1024×1024). This results in a resolution of 25×25 μm and 10 minutes acquisition time. Finally an IntraGate brightblood Cine FLASH sequence with retrospective gating was used to acquire cardiac images. The cardiac gating was derived from the saturation slice refocusing signal. IntraGate scans consisted of 5 slices with Tr=87.4 ms, Te=1.9 ms, FA=25°, ST=0.5 mm, FOV=25.6×25.6 mm, matrix size=512×512 and saturation ST=1 mm. 30 repetitions were recorded for retrospective reconstruction of 10 time points per slice. This results in a resolution of 50×50 μm and 11 minutes acquisition time. The tricaine mixture within the Eppendorf was refreshed by manual flushing after each different MR exam (sequence). MRI exams, including positioning of the zebrafish and coil adjustment, were performed within 35 minutes.

Results

In the new experimental setup the zebrafish were kept alive for at least 35 minutes in the MR magnet and they showed complete recovery thereafter.

High resolution images were acquired covering the complete zebrafish. An example of a high resolution FLASH image through the zebrafish is shown in Fig 2a, covering the brain, liver, swim bladder and heart. IntraGate cardiac images with 10 time points per slice were recorded, showing clearly the position of the heart and ventricular wall. An image of 1 time point, at the same position as the slice in 2a, showing the ventricular wall, is presented in Fig 2b. A histological slice of the zebrafish heart, oriented as the heart in the MR images and showing the atrium, ventricle and bulbus arteriosus (b.a.), is shown in 2c as reference.

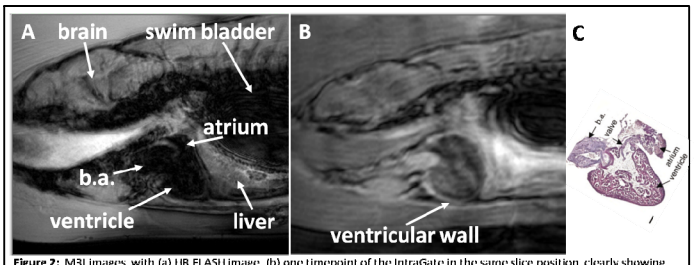

Figure 2: MRI images, with (a) HR FLASH image, (b) one timepoint of the IntraGate in the same slice position, clearly showing the ventricle wall and (c) a histological slice of the zebrafish heart oriented as in the MRI images

Conclusion and discussion

A new method to acquire high resolution MRI images of the living zebrafish with minimal movement artifacts was introduced. In addition we obtained the first *in vivo* cardiac images of time points of the heartbeat cycle in zebrafish using IntraGate brightblood Cine FLASH.

This technique can be of high value for investigating cardiac function in zebrafish models for cardiac disease as well as to study the regeneration of zebrafish heart *in vivo*. Furthermore, other immotile tissues, such as the brain, reveal an excellent signal quality due to the reduction of movement artifacts, suggesting that MRI can be an extremely valuable tool for the morphological and functional assessment of internal tissues and organs *in vivo* in zebrafish models.

References

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