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| RE: 2D and 3D echocardiography in the axolotl (*Ambystoma mexicanum*) |
| Cover letter |

Dear JoVE editor.

Please find attached our invited submission (invited by Science Editor Indrani Mukherjee, PhD, on June 6, 2017) of a JoVE protocol on 2D and 3D echocardiography in the axolotl. The axolotl salamander is a widely applied model species in regenerative biology and this species is increasingly being applied as a model for intrinsic heart regeneration. Contrary to the zebrafish, another well-established model in heart regeneration, the axolotl can attain sizes that allows for reproducible measurements of cardiac function. Since heart regeneration is ultimately a matter of restoring function rather than only cardiac anatomy, this renders the axolotl as an important cardioregenerative model for functional regeneration and recovery.

In this submission we strive to establish the methods to perform meaningful and reproducible echocardiography on the axolotl heart to evaluate function. We provide methods to measure cardiac function in both anesthetized (demonstrating three different anesthesia regimes) and unanesthetized axolotls using 2D echocardiography. We demonstrate how to measure and minimize subjectivity and operator bias in ultrasound measurements using intra/inter-operator/observer analysis. Finally, we establish a method to acquire three-dimensional echocardiographic data on the beating axolotl heart at a high spatiotemporal resolution. Our methods have been developed and refined in the axolotl, but could find uses in other amphibians as well (e.g. *Xenopus*)

We hope that you find this contribution of value to the readers/user of JoVE and are looking forward to communicate about the manuscript.

Kind regards

Henrik Lauridsen

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