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PSI SideView™ Imaging Microplate Application Note AN-04

Zebrafish Cardiac Output Assay

Doppler-Optical Coherence Tomography for Zebrafish Cardiac Assays

Objective

The overall objective of was to develop a non-invasive 3-D structural and functional imaging device for automated measurement of cardiac function in zebrafish larvae. The device, currently at the optical breadboard stage, enables clear visualization of atrial and ventricular contraction and precise vessel diameter and blood velocity measurement.

Introduction

Functional cardiac imaging is accomplished using Fourier Domain-Doppler Optical Coherence Tomography (FDODT). Low coherence techniques detect singly back-scattered light using an interferometer thus providing depth profiles of the tissue being probed. Measurement of Doppler broadening indirectly senses the flow velocity vector parallel to the incident beam. Use of a dual beam approach enabled absolute blood flow velocity measurement in a sagittal plane. The technology employs a near-infrared (NIR) broadband source. **Since zebrafish are insensitive to the source, it will not induce a light avoidance response, and will prevent stress-induced changes in heart rate.**

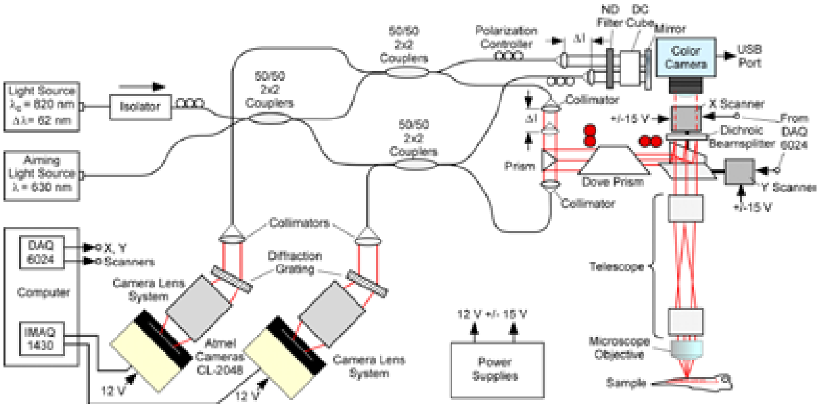

Figure 1.
Schematic of a dual-beam FDODT system

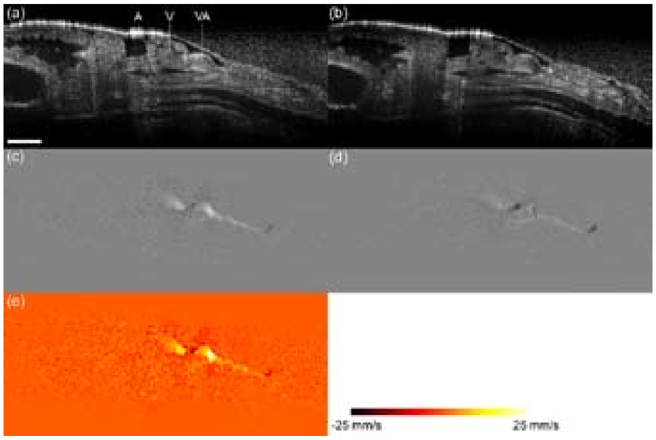

Figure 2.
Sagittal view of blood flow in the zebrafish heart. (a), (b) Intensity image in channels 1 and 2. (c), (d) Phase maps in channels 1 and 2. (e) Absolute velocity map. Locations of the atrium (A), ventricle (V), and ventral aorta (VA) are indicated. Scale bar = 100 µm. The animal is oriented ventral surface up and with its head to the right. The ventricle is clearly visible but the atrium is slightly offset from the plane of greatest flow and only a small portion is visible. Note the reversal of flow between V and VA caused by the closing of the aortic valve.

Methods

The system was designed as a compound microscope which required that the animals were immobilized in agarose and oriented ventral surface up. Larvae were first anesthetized using 50 µg/ml Tricaine. High-resolution video was collected at 18 frames per second (fps) and high-speed video was collected at 154 fps with reduced structural resolution. The next generation device will be designed as an inverted microscope and will not require anesthetization and immobilization of the larvae. Cardiac output was measured by integrating the mean velocity across the VA and multiplying by the cross sectional area, and the heart rate.

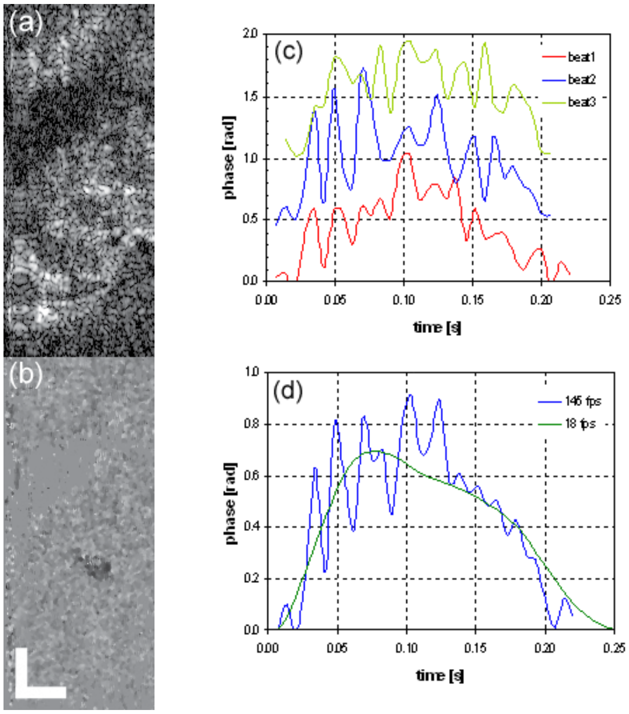

Figure 3.
Temporal phase profiles for high speed video. (a) Single intensity image for one channel (axial view). (b) Phase image for one channel. (c) Phase profile for three single beats. Curves are shifted laterally by 0.5 radians for clarity. (d) The average phase profile for the three beat shown in (c). The phase profile for a comparable video acquired at 18 fps is also shown. Scale bar = 50 µm

Results

The system provided up to 10 µm lateral and 6 µm axial resolution with a minimum detectable velocity of 0.3 mm/s at 18 fps. A typical heart rate of 157 bpm this provided 7 frames per heat beat in which flow in the VA was detectable in 3 frames. Peak flow approached 50 mm/s. The measured cardiac output for the animal depicted in Figure 2 was 174 nl/min with a stroke volume of 1.13 nl.

A comparison of phase profiles collected at 18 fps and at 154 fps yielded comparable integrated velocities (Figure 3). The high-speed phase data show a series of peaks that occur at fairly regular intervals for each of the three depicted cardiac cycles. These data demonstrate the utility of FDODT for measuring high-frequency changes that may increase understanding of cardiac physiology.

Conclusions

In conclusion, we have presented a new optical imaging tool, based upon FD-ODT, for use with wild-type and mutant zebrafish animal models. The technology has the potential to significantly enhance the capabilities of high-throughput phenotype-based screens of potential cardiac performance modifiers by providing high-resolution, three-dimensional structural and functional information, including flow velocity, stroke volume, cardiac output, and ejection fraction. The next step in system development is to enhance the instrument for automation necessary for high-throughput use.

Please inquire about your specific application.

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