

Dynamic Imaging and Analysis of Fast Cyclically Moving Embryonic Structures

Michael Liebling^{1*}, Arian S. Forouhar², Julien Vermot¹, Mory Gharib², Mary E. Dickinson³, and Scott E. Fraser^{1,2}

¹Biological Imaging Center, Beckman Institute, Caltech, MC 139-74, Pasadena, CA 91125, USA

²Option of Bioengineering, Caltech, Pasadena, CA 91125, USA

³Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030, USA

*Correspondence: liebling@caltech.edu

Biomechanical processes induced by blood flow contribute to the development of the embryonic heart [1]. The high speed at which they occur requires dynamic three-dimensional imaging at correspondingly high spatial and temporal resolution for study. Although confocal microscopy is the tool of choice for imaging live biological samples in depth, direct three-dimensional imaging at the required frame-rate (typically in the range of 60 to 200 frames per s) is not currently possible. However, recently developed confocal microscopes allow the capture of hundreds of two-dimensional optical sections per second. Therefore, when the motion of the imaged objects is cyclic, it is possible to acquire slice sequences at fixed depths in the sample, retrospectively synchronize them, and finally build a dynamic three-dimensional volume.

We developed automatic synchronization procedures to allow for a fast, reproducible, and scalable workflow. The latter are capable of handling the considerable amount of data, typical fluorescence imaging caveats (e.g. low photon count and photo-bleaching) and motions that are not strictly periodic via a nonuniform temporal registration approach. The algorithms rely on the minimization of the pixel intensity difference between adjacent slice-sequence pairs, a criterion that we express in the wavelet domain and which leads to an efficient and stable implementation [2,3].

In the example shown in Fig. 1, we spawned wild-type zebrafish (*danio rerio*) using standard techniques [4] and imaged a 38 hours post fertilization (h.p.f) old embryo that was soaked in a green fluorescent dye (BODIPY FL C₅-ceramide, Molecular Probes) for 3 hours. We acquired two-dimensional image sequences at 60 frames per s for 2 s using a fast slit-scanning confocal microscope (Zeiss LSM 5 LIVE, Carl Zeiss Jena GmbH, Germany) with 488nm excitation light and a 500–525nm band-pass filter to collect the emission signal. Each sequence covered 3–4 heartbeats and was performed at a fixed depth. We repeated the acquisition after moving the stage vertically by 5 μm . Typically we acquired sequences at 20–30 depths. Since the acquisition was nongated, the slice sequences are not synchronized. We then realigned the images by use of a MATLAB implementation of the wavelet-based synchronization algorithm. Finally, we visualized the four-dimensional data using appropriate software (Imaris, Biplane AG) and extracted such information as blood velocity, heart wall motion patterns, and volume changes.

The proposed approaches for in vivo imaging of the embryonic zebrafish heart should contribute to reach a better understanding of the mechanisms that drive heart development.

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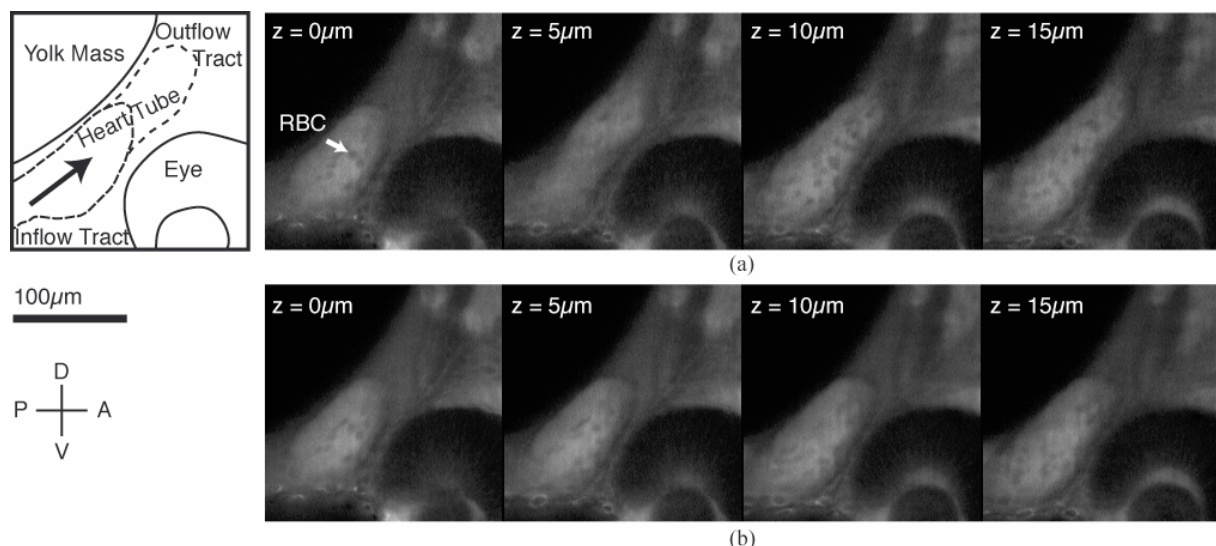


FIG. 1. Single time-point of a beating 38 hpf embryonic zebrafish heart tube. (a) Confocal slices acquired sequentially at 5 μm -spaced vertical positions (Zeiss C-Apochromat 40 \times /1.2W microscope objective). The frame acquisition time was 1/60 s. Since the acquisition is nongated, images at different depths correspond to arbitrary moments in the cardiac cycle. Red blood cells, which are not fluorescent, correspond to dark spots (RBC). (b) After temporal synchronization of the slice sequences, each slice at a given time-point corresponds to the same moment in the cardiac cycle.