Improving specificity in Bright-Field Microscopy Images of the Beating Embryonic Heart via Motion-Based Separation

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Summary: High-speed, bright-field (BF) microscopy of the beating embryonic zebrafish heart reveals both static background structures as well as the rapid motion of cardiac tissues and red blood cells (RBCs). However, all structures contribute to BF image contrast, making labeling and subsequent analysis of these images difficult. We review our progress to separate cardiac BF images into three separate channels, based on the periodic, aperiodic, or static motion patterns respective to each of the cardiac structures. We show that the three extracted channels can be recombined into a pseudo-colored 3D image sequence. We anticipate that this imaging technique will facilitate quantitative characterization of heart function during normal and abnormal cardiac development.

Fluorescence microscopy (FM) and BF microscopy are two popular in-vivo microscopy techniques for capturing the heart development in embryonic zebrafish. FM produces images that have structure-specific contrast, with the different parts of the heart distinctly labeled. However, due to the low photon counts in FM, only frame rates of about 30 full frames per second (fps) are typically achievable (on high-end electron-multiplier charge coupled device EM-CCD cameras), which is insufficient to capture the dynamics of the heart without motion artifacts like aliasing or blurring. BF on the other hand enables imaging at thousands of frames per second but the images lack tissue-specific contrast (Fig. 1(a)).

![Fig. 1: a) Raw bright-field microscopy image showing non-specific contrast, making heart-wall, RBCs and static background structures hard to distinguish. b) Frame resulting from the separation of sequence (a), showing the RBCs in yellow and the heart-wall in red, yielding images that have both high frame-rate and tissue specific contrast.](image)

Here we review our progress [1,2,3] in developing a technique that improves the specificity of BF cardiac images, by separating the input sequence into periodic, aperiodic, and static structures based solely on their motion characteristics, via temporal registration of image sequences acquired over multiple heartbeats. The resulting sequences are pseudo colored to produce a multi-color visualization with a higher degree of specificity than the input images (Fig. 1(b)). The separation effectiveness of the technique is first quantified by applying the technique to a synthetic heart-beat dataset. Finally, results on actual BF data are presented.