

# Simultaneous Screening of Zebrafish Larvae Cardiac and Respiratory Functions: A Microfluidic Multi-Phenotypic Approach

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## Abstract

Multi-phenotypic screening of multiple zebrafish larvae plays an important role in enhancing the quality and speed of biological assays. Many microfluidic devices have been presented for zebrafish phenotypic assays, but multi-organ screening of multiple larvae, from different needed orientations, in a single device that can enable rapid and large-sample testing is yet to be achieved. Here, we propose a multi-phenotypic quadruple-fish microfluidic chip for simultaneous monitoring of fin movement and heart activity of 5–7-day postfertilization zebrafish larvae trapped in the chip. In each experiment, fin movements of four larvae were quantified in the dorsal view in terms of fin beat frequency (FBF). Positioning of four optical prisms next to the traps provided the lateral views of the four larvae and enabled heart rate (HR) monitoring. The device's functionality in chemical testing was validated by assessing the impacts of ethanol on heart and fin activities. Larvae treated with 3% ethanol displayed a significant drop of 13.2% and 35.8% in HR and FBF, respectively. Subsequent tests with cadmium chloride highlighted the novel application of our device for screening the effect of heavy metals on cardiac and respiratory function at the same time. Exposure to 5 µg/L cadmium chloride revealed a significant increase of 8.2% and 39.2% in HR and FBF, respectively. The device can be employed to improve quantitative multi-phenotypic screening of zebrafish larvae in response to chemical stimuli in various chemical screening assays, in applications such as ecotoxicology and drug discovery.

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