COPAS™ Application Note XL-02

D. rerio Analysis and Sorting: High throughput screening of innate immune responses in zebrafish embryos

Profiling of live *D. rerio larvae* using the COPAS XL instrument

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Objective

Live *D. rerio* larvae, injected with mCherry transfected mycobacteria were analyzed and sorted using the COPAS XL instrument (Union Biometrica). The larvae showing granuloma formation were dispensed into 96 well microtiter plates and analyzed by microscopy.

Introduction

Multiple applications can be pursued using the COPAS XL instrument (See Figure 1.) The COPAS XL is the largest flow cell instrument in the COPAS product line and has been used for evaluation of third instar *Drosophila*, *Daphnia*, *Oryzias latipes* (medaka) and *Xenopus* sorting applications. The COPAS XL is a multiparameter flow cytometer that enables the user to distinguish and sort discrete populations using size, optical density and fluorescence parameters. Objects passing the flow cell can be analyzed using the Profiler II option, showing optical density and fluorescence profiles for each individual larva (See Figure 2). This application involves sorting live zebrafish larvae using size (Time Of Flight=TOF), optical density (Extinction=EXT) and Fluorescence Profiles.

Figure 1. The COPAS *XL*

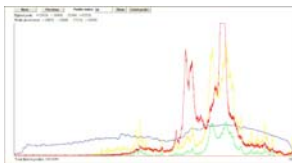

Figure2. Zebrafish Profile: The blue line represents optical density of the larva. The green, yellow and red lines represent profiles of fluorescence detection.

Materials

COPAS *XL* (Union Biometrica, pn 370-5000-000), equipped with 488 nm and 561 nm solid state lasers and Profiler II Option
Leica stereo microscope

Method

The injection was performed on Fli1-GFP Zebrafish embryos at 48hpf (2 days post fertilization). Stably transformed mCherry mycobacteria (+/- 20000) were injected into the yolk. The bacteria were suspended at the appropriate concentration. The analysis was done at day 5 post fertilization.

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Analysis using the COPAS XL:
Batches of injected embryos were added to the sample cup and analyzed using a 488 nm and 561 nm laser. Size (TOF) and optical density (EXT) were acquired using the 488 laser. The mCherry was excited by both lasers (8% from 488 nm laser and 64% from the 561 nm laser). Emission of the mCherry was collected using a 615/24 nm band pass filter. Analyzed fish were sorted at 1 per well into a 96 well plate for microscopic analysis. Microscopic images were acquired using a Leica stereo microscope, model MZ 16 FA equipped with a DsRed filter.

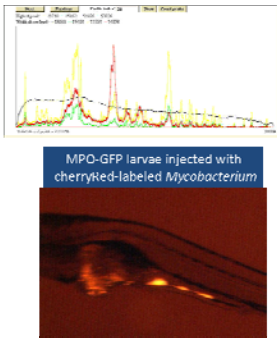

Figure 3. Image of a *D. rerio* embryo and corresponding profile (before compensation of spectral overlap).

PROFILING

Profiler II digitizes the object into a succession of peaks and valleys that directly trace the optical density and fluorescence intensity of the object as it passes through the flow cell. Profiling allows to sort based on the peak height, peak width, number and location of peaks within the larvae (Figure 2).

Results

Sorted larvae were compared using the fluorescence image and profile (Figure 3). GFP emission and autofluorescence, generated from the excitation of the 488 laser cause spectral overlap into the PMT that detects mCherry emission (visible by the green and yellow line in Figure 3). Electronic compensation allows eliminating the spectral overlap and displaying the emission from the Red PMT only (See Figure 4). The COPAS XL also allows generating profiles for Extinction and Red Fluorescence using the 561 nm Solid State laser alone (See figure 5).

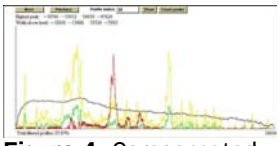

Figure 4. Compensated profile (Red-50% Yellow) to eliminate spectral overlap.

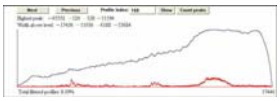

Figure 5. Optical density and weak mCherry emission profile generated using the 561 nm laser alone.

Discussion

In recent years the zebrafish has been shown to be an excellent model for studying the mechanisms of the innate immune defense against pathogens. Previous studies have shown that transcriptome responses towards pathogens such as *Mycobacterium marinum* and *Salmonella typhimurium* are very similar to responses in mammalian systems. Using combinations of transcriptomic deep sequencing, morpholino knockdown and transgenic reporter fish technologies we have obtained new insights in the functions of key players of the innate immune system. These results are not only relevant to infectious diseases but also to the study of immune responses to cancer cells, for instance using xeno-transplantation assays. Here we have shown that such studies can also be extended to a high throughput level. Using the COPAS XL, large numbers of injected fish (using a automated injection system) can be analyzed in an unbiased manner and sorted for screening into multi-well plates.

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