UNION BIOMETRICA
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COPAS™ Application Note XL-01
Zebrafish Sorting: Live embryos vs. Dead eggs

Automated Detection and Sorting of live *D.rerio* embryos from dead *D.rerio* eggs using the COPAS *XL* instrument

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Objective

Live *D.rerio* embryos were detected and sorted from mixed populations of live embryos and dead eggs usings the COPAS *XL* instrument. These embryos were dispensed into 96 well microtiter plates and then scored for accuracy, purity, and viability.

Introduction

Multiple applications can be persued using the COPAS *XL* instrument (See Figure 1). The COPAS *XL* is the largest flow cell instrument in the COPAS product line and has also been used for evaluation of third instar *Drosophila* larvae and for large bead sorting applications. The COPAS *XL* is a multiparameter flow cytometer that enables the user to distinguish and sort discrete populations using size and fluorescence parameters. As a first step demonstration of Union Biometrica's capabilities, this application involves sorting live zebrafish embryos from dead zebrafish eggs using two size parameters, Time of Flight (TOF) and Extinction (EXT). Time of Flight is an indicator of the length, and Extinction is an indicator of the size and internal structure of the multicellular organism.

Figure 1. Picture of the COPAS *XL* System

Materials

COPAS *XL* (Union Biometrica, pn 370-5000-000)
Standard fish* water with methylene blue as a fungicide
96 well microtiter plates filled with 150 µm of fish water/well
Dissecting light microscope

Method

Mixed populations of wildtype zebrafish embryos and dead eggs were evaluated using the COPAS *XL*. All samples were diluted with standard fish water to obtain a final concentration of 25 animals/mL prior to processing on the COPAS *XL*. The zebrafish embryos were between 10 and 14 hours post fertilization when analyzed. The animal suspension was placed into the COPAS *XL* sample cup and analyzed using the size and optical parameters, TOF and EXT. COPAS is also capable of sorting on multiwavelength fluorescence (either transgenic fish or fish stained with a fluorescent dye). This will be demonstrated in a future applications note. Two distinct populations were observed on the TOF vs. EXT dotplot (See Figure 2). The left population contained live embryos. The right population contained dead eggs.

The live embryo region was chosen for sorting. Multiple 96 well plates were filled with 1 to 3 live zebrafish embryos per well. Sorting accuracy (number of wells filled versus empty) was assessed by microscopy. 24 hours after sorting, a subset of plates were scored to evaluate the impact of processing on the viability of the embryos. Figure 3. shows a sorted live embryo and a sorted dead egg for comparison.

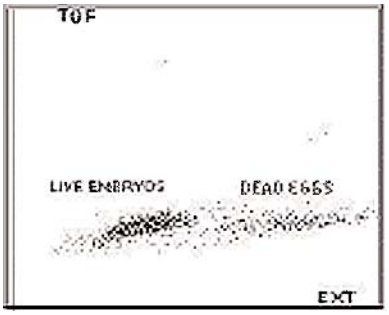

Figure 2. Image of a mixed population of D. rerio embryos.

Results

See Table 1 for compiled results.

SORTING ACCURACY: 3,097 zebrafish eggs were sorted into 96 well microtiter plates. One to three eggs were sorted into each well. These were viewed using a light microscope to determine how many wells were filled or not filled with eggs. Of the 3,097 wells, 3,079 contained eggs and 28 wells were empty. The sorting accuracy of the instrument was 99.4%.

SORTING PURITY: 2,027 live zebrafish embryos were sorted into 96 well microtiter plates from mixed populations of live embryos and dead eggs, The wells were viewed using a light microscope to determine how many wells were filled with live embryos and how many contained dead eggs. Of the 2,027 wells filled, 2,003 contained live embryos and 24 contained dead eggs. The sorting purity was 98.8%.

VIABILITY: 471 wells filled with live zebrafish embryos were viewed using a light microscope post sorting to determine the impact of processing on the viablitiy of the embryos. Of the 471 embryos dispensed, 446 (94.7%) of the live embryos sorted remained viable.

	Total Number Evaluated	Positive Results	%
Sorting Accuracy	3,097	3,079	99.4
Sorting Purity	2,027	2,003	98.8
Viabilty	471	446	94.7


a.

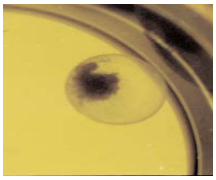
b.

Figure 3. Image of: a.) sorted live D. rerio embryo b.) dead D. rerio egg.

Conclusions

These data show that dispensing and sorting (based on size and optical parameters) of live *D.rerio* embryos from a mixed population can be performed successfully with the COPAS *XL*. Furthermore, viability of the zebrafish embryos remains high (94.7%) after the procedure.

References

*The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Danio rerio*). University of Oregon Press, Eugene, OR. Edition 4, 2000.