UNION BIOMETRICA
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COPAS™ Application Note B-05

C.elegans Dual Color Fluorescence Sorting

Automated Detection and Sorting of Living *C.elegans* from a Mixed ZsGreen and Propidium Iodide Labeled Population

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Objective

C.elegans*, labeled with both ZsGreen** (Green Coral Reef Protein, Clontech) and Propidium Iodide (PI, Molecular Probes Inc.) were used for sorting living ZsGreen positive worms from a mixed population. The COPAS *BIOSORT* is capable of dual fluorescence analysis and sorting, using a 488-nm laser excitation and two Photo Multiplier Tubes for fluorescence detection.**

Figure 1. The COPAS *BIOSORT* was used to analyze and sort *C.elegans*.

Introduction

The COPAS *BIOSORT* is a high throughput system that analyzes and sorts *C.elegans* based on physical and optical parameters (figure 1). In this application, two fluorescent reporters were used, ZsGreen and PI. PI binds by intercalating with DNA, but dye uptake only occurs with dead cells (Shapiro, 1995). Transgenic worms were prepared, with ZsGreen expressed in the pharyngeal muscle. This application was designed to show that a live v. dead stain may be used on fluorescently-tagged organisms, for COPAS Technology discrimination between living and dead worms.

The COPAS *BIOSORT* is equipped with two lasers. The 633-nm excitation laser is used to analyze physical parameters of the organism. Time of Flight (TOF) displays the relative length of an organism and Extinction (EXT) provides a measurement of its optical density.

The 488-laser is used to excite both ZsGreen and PI. The optical system of the COPAS allows simultaneous excitation and separate collection of two fluorescence emission parameters. In this experiment, FLU1 (498-522 nm) corresponds to ZsGreen, and FLU2 (575-595 nm) to PI.

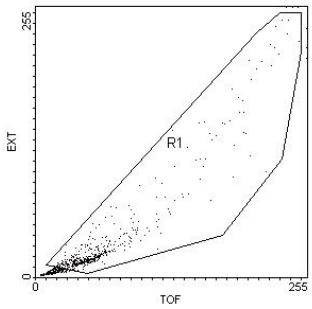

Figure 2. Image of the TOF (length) and EXT (density) of *C.elegans* in a mixed population. R1 was used to eliminate analysis of debris and eggs.

Materials

COPAS *BIOSORT* (Union Biometrica, pn 350-5000-000)
M9 buffer with 0.01% Triton X-100
50 mL conical tubes
Propidium Iodide (Molecular Probes, P-3566)
C. elegans organisms expressing ZsGreen protein

Method

We started with a population of ZsGreen expressing worms. Samples were prepared individually by washing the worms off a plate using M9 buffer. The worms were collected in two 50 ml tubes (SEE SAMPLE PREPARATION PROTOCOLS SP01and SP02). The first ZsGreen expressing sample was placed into the COPAS *BIOSORT* sample cup. Two size parameters, Time Of Flight (TOF, length) and Extinction (EXT, optical density), were used to analyze the population.

A gating region (R1) was drawn on an EXT versus TOF dot plot (figure 2) to eliminate eggs or debris. The sorting dot plot was set so that the FLU1 (ZsGreen signal) and FLU2 (PI signal) parameters were displayed. The dot plot in figure 3 shows FLU2 versus FLU1 for the ZsGreen population without PI staining. The ZsGreen sample was used to establish a sort region (R2) for PI -stained (dead) worms.

The second ZsGreen expressing population was heat shocked for 5 minutes at 60°C. PI was added (10µL per mL worm suspension) and incubated for 15 minutes.

Both heat-treated and non-treated samples were mixed and analyzed. Figure 4 shows the FLU2 versus FLU1 dot plot for the ZsGreen population stained with PI. PI -stained worms (2.62%) were sorted and re-analysed to verify the sort performance.

After confirmation of the sort performance, R3 was set on the PI-free population for sorting of living ZsGreen expressing worms (figure 5). The viability of worms in sort region R3 was confirmed.

Results

Statistical results of the two regions:
Total Events 1260;
Gated Events 544 43.17%
System: Log Parameter Means: Geometric

Region	Events	%Total	%Gated
R1	544	43.17	100.00
R2	87	6.90	15.99
R3	360	28.57	66.18

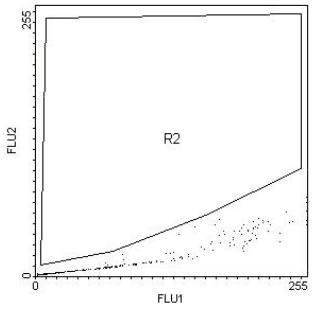

Figure 3. Dot plot of the ZsGreen fluorescent *C.elegans* population without PI.

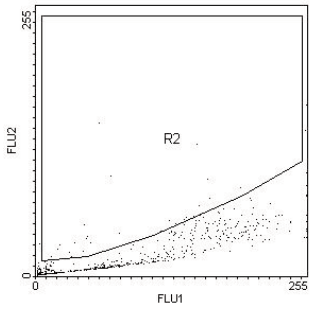

Figure 4. Dot plot of the ZsGreen *C.elegans* population stained with PI.

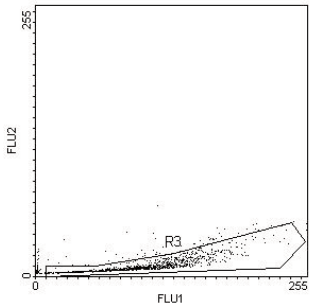

Figure 5. Region R3 was created to sort viable (PI-free) ZsGreen *C.elegans*.

Discussion

This application illustrates the capability of the COPAS *BIOSORT* system to differentiate and sort distinct worm populations by dual fluorescence analysis. In this example, a red fluorescent stain (Propidium Iodide) was used on ZsGreen fluorescent worms to differentiate dead from living animals and selectively sort the viable worms.

The choice of optical filters in the COPAS system allows analysis of two colors simultaneously. Overlap of the spectra in two color combinations can prevent correct analysis of positive events. In this case, a sort confirmation was done on the double positives (Region R2). The result indicates that spectral overlap between ZsGreen and PI is minimized, so both populations can be clearly identified and sorted.

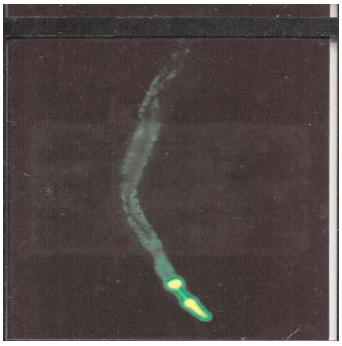

Figure 6. Image of a sorted living ZsGreen *C.elegans*

Reference

WinMDI, Joe TrotterScripps Institute, San Francisco**
ZsGreen kindly provided by Clontech;
For Research Use Only*** Molecular Probes Inc.
Shapiro, H. (1995)
Practical Flow Cytometry.
Wiley-Liss, Inc.

Figure 7. Image of a dead (PI-stained) ZsGreen *C.elegans*.