COPAS™ Application Note B-06

C.elegans Dual Color Fluorescence Sorting

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

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Objective

C.elegans, labeled with both ZsYellow (yellow coral reef protein; Clontech) and Propidium Iodide (PI; Molecular Probes, Inc.) were used for sorting living ZsYellow positive worms from a mixed population. The COPAS *BIOSORT* is capable of dual fluorescence analysis and sorting, using a 488 nm excitation laser 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, ZsYellow and PI. PI binds by intercalating with DNA but dye uptake only occurs with dead cells (Shapiro, 1995). Transgenic worms were prepared, with Zs yellow expressed in the pharyngeal muscle. This application was designed to show that a live vs. dead stain may be used on fluorescently-tagged organisms for COPAS Technology discrimination between living and dead worms.

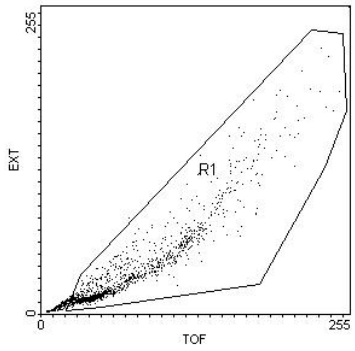

Figure 2. Dot plot of TOF (length) and EXT (density) of *C.elegans* in a mixed population.

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) is a measure of relative length of each organism, and Extinction (EXT) provides a measurement of its optical density. The 488 nm laser is used to excite both ZsYellow and PI.

The optical design of the COPAS allows simultaneous excitation and separate collection of two separate fluorescent reporters. In this experiment, FLU1 (530-560 nm emission) corresponds to ZsYellow, and FLU2 (575-

595 nm emission) to PI. The optical emission filters designed for the system prevent spectrum overlap and allow for clear resolution between ZsYellow and PI.

Materials

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

Method

We started with a population of ZsYellow 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 ZsYellow 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 (ZsYellow signal) and FLU2 (PI signal) parameters were displayed. The dot plot in Figure 3 shows FLU2 versus FLU1 for the ZsYellow population only. The ZsYellow sample was used to determine the region (R2) for PI stained (dead) worms.

The second ZsYellow expressing population was heat shocked for 5 minutes at 60°C. PI was added (10 µL PI per ml of worm sample) and incubated for 15 minutes. Both samples were mixed and analyzed. Figure 4 shows the FLU2 versus FLU1 dot plot for the ZsYellow population stained with PI. PI positive worms (1.63%) were sorted and re-analysed to check the sort performance

After visual confirmation of the sorting performance, R3 was set on the PI-free population for sorting of living ZsYellow expressing worms. (Figure 5)

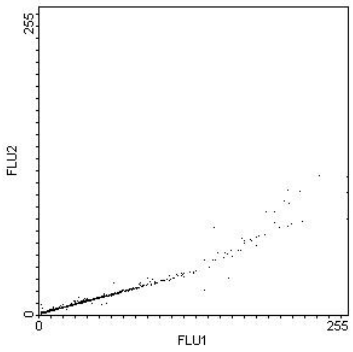

Figure 3. Dot plot of the ZsYellow fluorescent *C.elegans* population without PI.

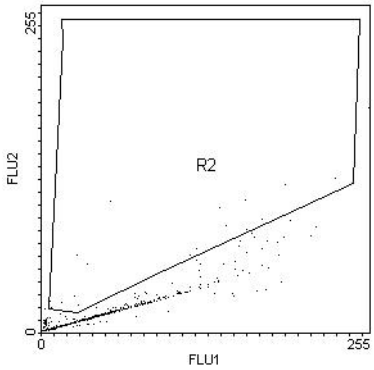

Figure 4. Dot plot of the ZsYellow *C.elegans* population stained with PI.

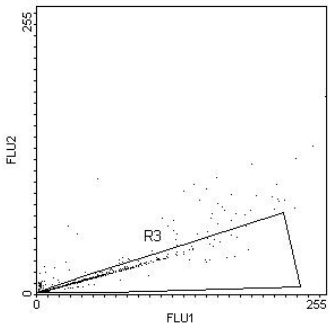

Figure 5. Region R3 was created to sort viable (PI-free) ZsYellow positive worms.

Results

Figure 6 shows the microscopic analysis of the living ZsYellow population that was sorted.
Figure 7 shows a ZsYellow/PI dual-labeled worm.

Statistic results (WinMDI software):
Total Events 1291 Gated Events 697 53.99%

Region	Events	%Total	%Gated
R1	697	53.99	100.00
R2	61	4.73	8.75
R3	597	46.24	85.65

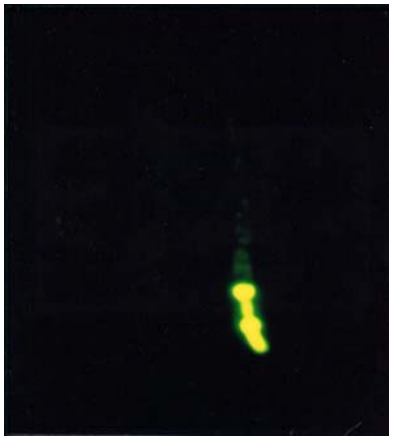

Figure 6. Image of the ZsYellow positive sorted *C.elegans*.

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 ZsYellow fluorescent worms to differentiate dead from living animals, and to selectively sort out the living 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 ZsYellow and PI is minimized, so both populations can be clearly identified and sorted.

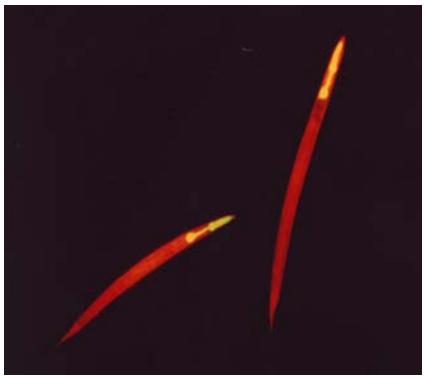

Figure 7. Image of a dead (PI positive) ZsYellow stained *C.elegans*.

Reference

Clontech, Palo Alto, CA.

Molecular Probes, Inc.

Scripps Institute, San Francisco

Shapiro, Howard. (1995) *Practical Flow Cytometry*. Wiley-Liss, Inc.

WinMDI, Joe Trotter