UNION BIOMETRICA
A Harvard Bioscience Company

COPAS™ Application Note B-03

C.elegans SORTING BY DEVELOPMENTAL STAGE

Automated Detection and Sorting of *C.elegans* at Different Development Stages From a Mixed Population

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Objective

The objective of this experiment was to demonstrate that *C.elegans* at different development stages can be selectively sorted from a mixed population. The COPAS *BIOSORT* selects only those of the desired stage of development and places them into a stationary bulk container or individually into microtiter wells.

Figure 1. The COPAS *BIOSORT* was used to dispense 3,000 *Cl.elegans* of a certain stage into a tube in 8 minutes.

Introduction

The COPAS *BIOSORT* is a high throughput system that analyzes and sorts *C.elegans* using size and fluorescence parameters (Figure 1). For this application, two size parameters are used, 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 multi-cellular organism.

Materials

COPAS *BIOSORT* (Union Biometrica, pn 350-5000-000)
Liquid *C.elegans* culture medium
M9 buffer with 0.01% Triton X-100
15 mL conical tubes
Bright field microscope with 35 mm camera

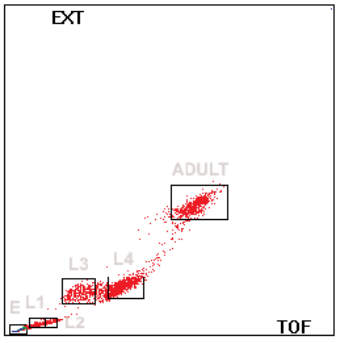


Figure 2. Image of the different stages of *C.elegans* in a synchronized population.

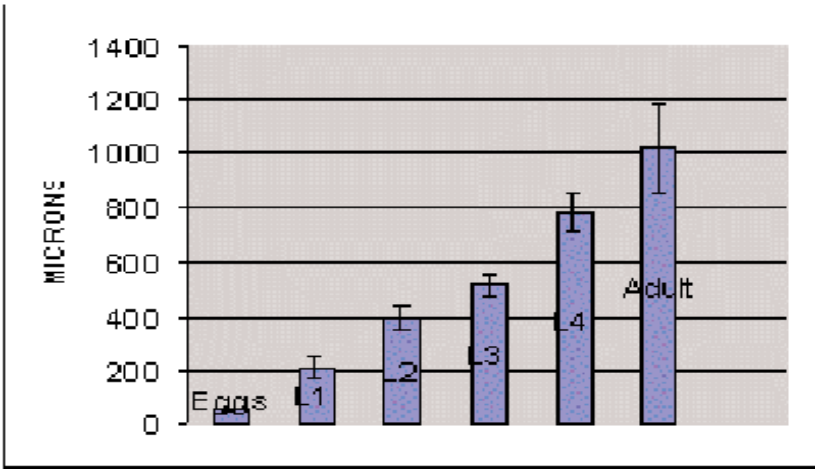
Method

We started with a large mixed population of N2 worms, with relatively even numbers of organisms at different developmental stages. For these experiments, a liquid culture was used. Clumps of embryos and debris were removed by passing the liquid through a nylon mesh **(SEE SAMPLE PREPARATION PROTOCOLS SP01 and SP02)**. The suspension was placed into the COPAS *BIOSORT* sample cup. Two size parameters, Time Of Flight (TOF) and Extinction (EXT), were used to analyze the population.

Sorting regions were chosen on a TOF versus EXT dot-plot (Figure 2). A number of animals were collected and analyzed to determine the appropriate regions for sorting of a particular stage of development. Once a region was selected, worms were dispensed into tubes (3,000 in each tube in 8 minutes). After collecting a sufficient number of worms of one particular stage in a tube, the sort gate was changed in order to select a different stage. This procedure was continued for the isolation of each of the different stages of the life cycle.

The sorted animals were transfered to slides and photographed using a 35 mm camera mounted to a bright field microscope. The worms were either measured dirsctly from the prints or were first scanned and measured using software. Embryos were used to calibrate the instruments.

Results



The graph, above, shows the mean sizes of all the animals sorted from each region. Error bars were calculated using standard deviation. All stages were visually confirmed.

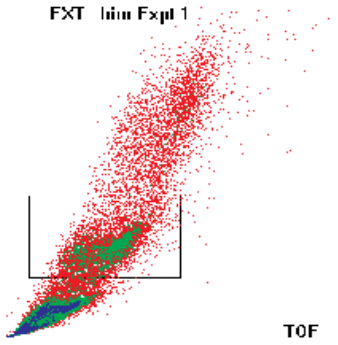


Figure 3. Dot Plot of the different stages of *C.elegans* in a non-synchronized population.

Discussion

This application shows the ability to sort different stages of an unstained worm population with the COPAS *BIOSORT* system using length and internal complexity. These two parameters are sufficient to identify 6 different development stages. Eggs are clearly separated. L1 and L2 are not distinguished in EXT, but can be separated by using TOF. L3 and L4 also do not have significant differences in EXT, but are clearly separated by TOF. The adults are significantly larger and have more internal complexity. These data demonstrate that specific developmental stages of *C.elegans* can be sorted with a high degree of purity using the COPAS *BIOSORT.*