Analysis and sorting of C. elegans based on peak counting of GFP expressing seam cells

Objective

The purpose of this experiment was to test the feasibility of using the BioSorter instrument to analyze C. elegans worms containing GFP positive seam cells and to demonstrate the ability to discriminate individual worms with altered seam cell numbers.

Introduction

The COPAS family of research instrumentation comprises a collection of large particle cytometers utilizing at least 1 excitation laser and up to 8 channels of fluorescence collection. Unique to COPAS instrumentation is the Profiling feature which graphically plots the fluorescence intensity changes along the length of the object as it passes through the laser(s). Large objects up to 1.5mm in diameter can be analyzed for physical and fluorescence characteristics and gently dispensed into a multi-well plate or other collection container for further investigation or reuse. The COPAS Vision also has equipped a camera to take an image of the object inside the flow channel. This image accompanies the cytometry data and can be analyzed using Union Biometrica's FlowPilot software or other image analysis tools.

In this experiment, the capabilities of the Profiling feature were utilized to identify and count fluorescence emission from a GFP reporter in the C. elegans seam cells. In order to score seam cell numbers in the nematode C. elegans, transgenic strains were used that expressed a nuclear seam cell marker (scm::gfp) in conjunction with a non-exclusively nuclear scm::rfp reporter. Seam cells fuse at the last larval stage (L4). Worms can be sorted by size to obtain late L4 - adult animals and the non-nuclear distribution of scm::rfp used to confirm that the whole seam length is visible to the detector (i.e. the worm is not twisted). The number of scm::gfp positive nuclei (green) can subsequently be scored. Adult wild type worms possess 16 seam cells per side; such worms are represented by strain AW567 (genotype; unc119(ed3); ouEx246 [unc-119(+) + scm::gfp + scm::rfp]. Strain AW568 expresses the same fluorescent reporters, but additionally carries a transcription factor overexpressing construct which gives rise to additional seam cells (genotype; unc119(ed3); ouEx247 [unc-119(+) + scm::gfp + scm::rfp + pAW508]

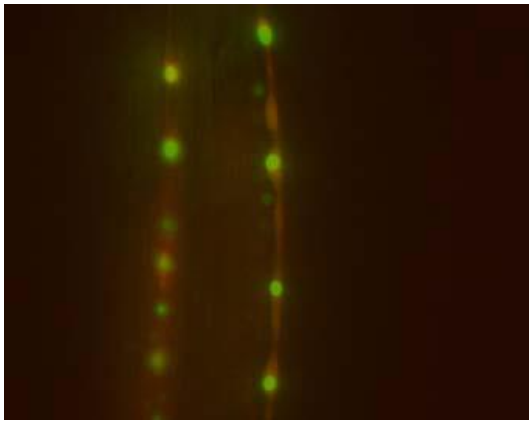


Figure 1. *AW 567*

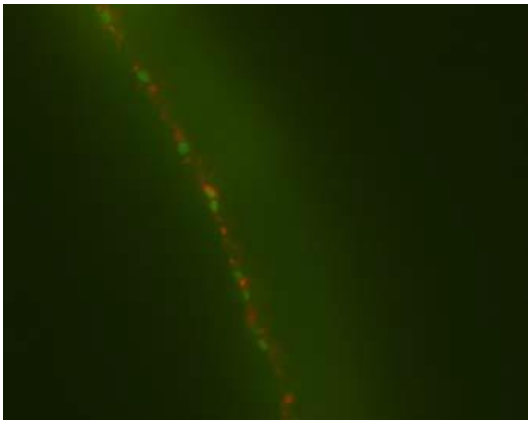


Figure 2. *AW 568*

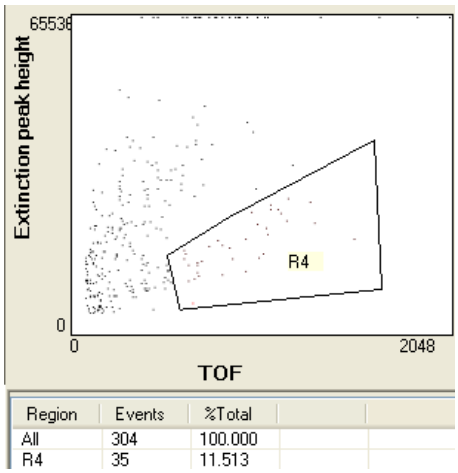
Materials and Methods

The BioSorter instrument, equipped with the 250 micron FOCA (Fluidic and Optical Core Assembly) is able to analyze and sort large objects (10-170 microns diameter) on the basis of the physical characteristics of size (TOF), optical density (EXT), and fluorescence signals (FLU). Objects are passed axially, one by one, through the focus of a laser beam. Relative size is determined by the time of flight (TOF) measurement. The optical density of the object is determined by the extinction (EXT) measurement. Objects can be dispensed into microtiter plates or stationary receptacles. Using a unique, pneumatic sorting mechanism, the BioSorter is gentle enough to sort and dispense live organisms without affecting the viability.

The BioSorter instrument was equipped with a 488 nm solid state laser for size, density and GFP excitation and a 561 nm solid state laser for excitation of the RFP. Emission filters were a BP510/23 and BP615/25 nanometer.

Worms were washed off agar plates using cold (4ºC) M9 solution and collected in a 50 ml conical tube. The plates had been kept at a minimum of 1 hour at 4ºC. Acquisition was performed at a speed of 10 worms per second. Cold M9 was used to prevent the worms from moving in the flow cell to optimize the correct detection of the peaks.

Results



Region	Events	%Total
All	304	100.000
R4	35	11.513

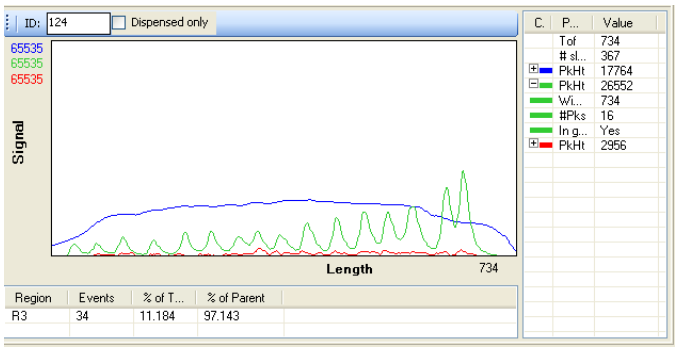


Figure 3 *At left TOF and EXT dot plot used for gating (R4) to select L4 – Adult worms. The right Profiler graph shows the distribution of GFP (green) peaks and RFP (red) over the axis of the worm. The blue line represents the optical density profile. The horizontal axis represents the TOF (size) of the worm.*

Sort Results and scoring of peaks

Worms from the two samples were selected based on peak counting using the BioSorter profiler definitions and dispensed at 1 per well in a 96-well plate.

Two plates were sent to Peter Appleford in Oxford for imaging and manual peak counting verification. The two lateral seam syncytia represent independent lineages and it is not possible to know which side is read by the detector. However, worms overexpressing pAW508 typically exhibit seam hyperplasia on both sides.

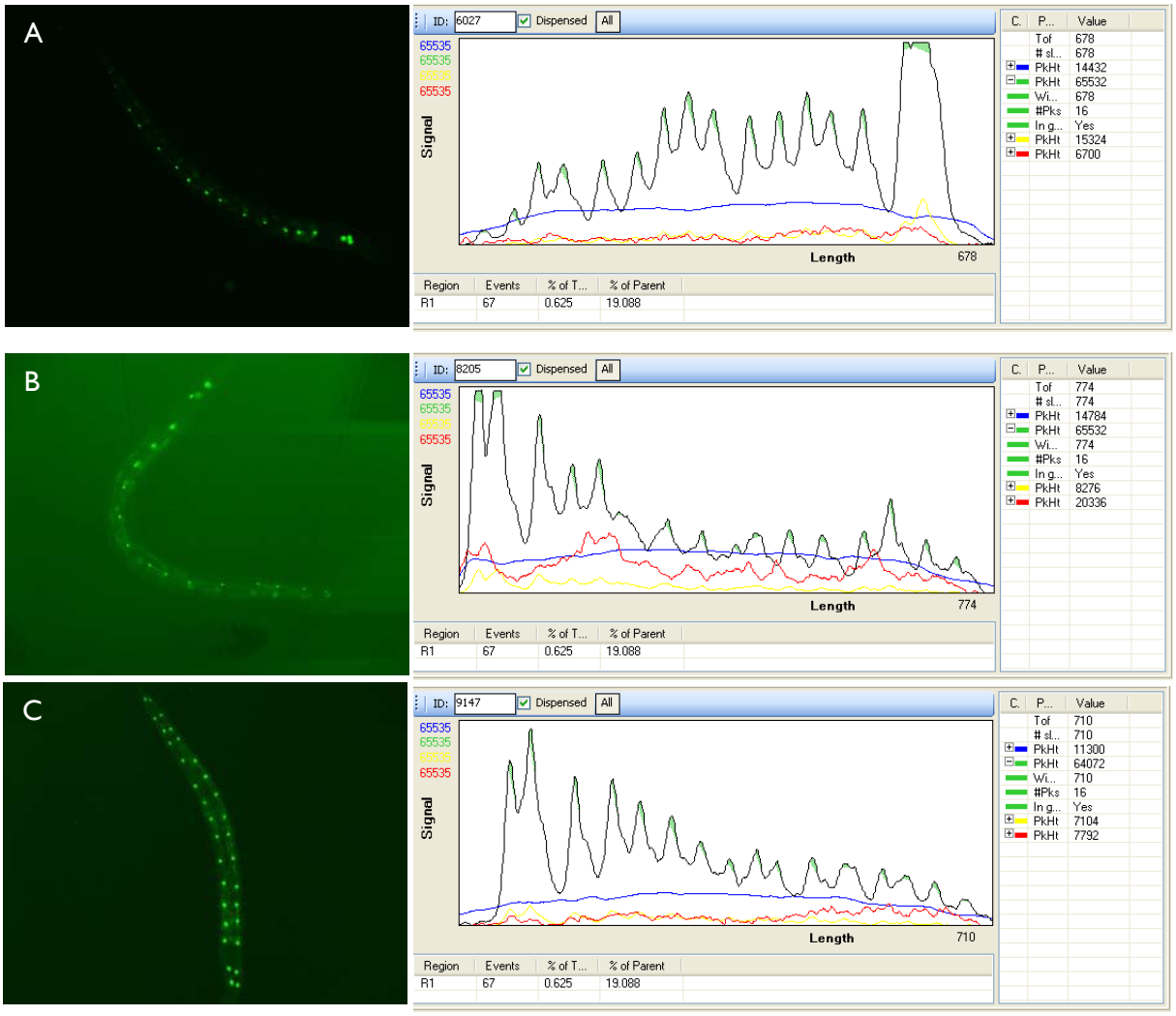
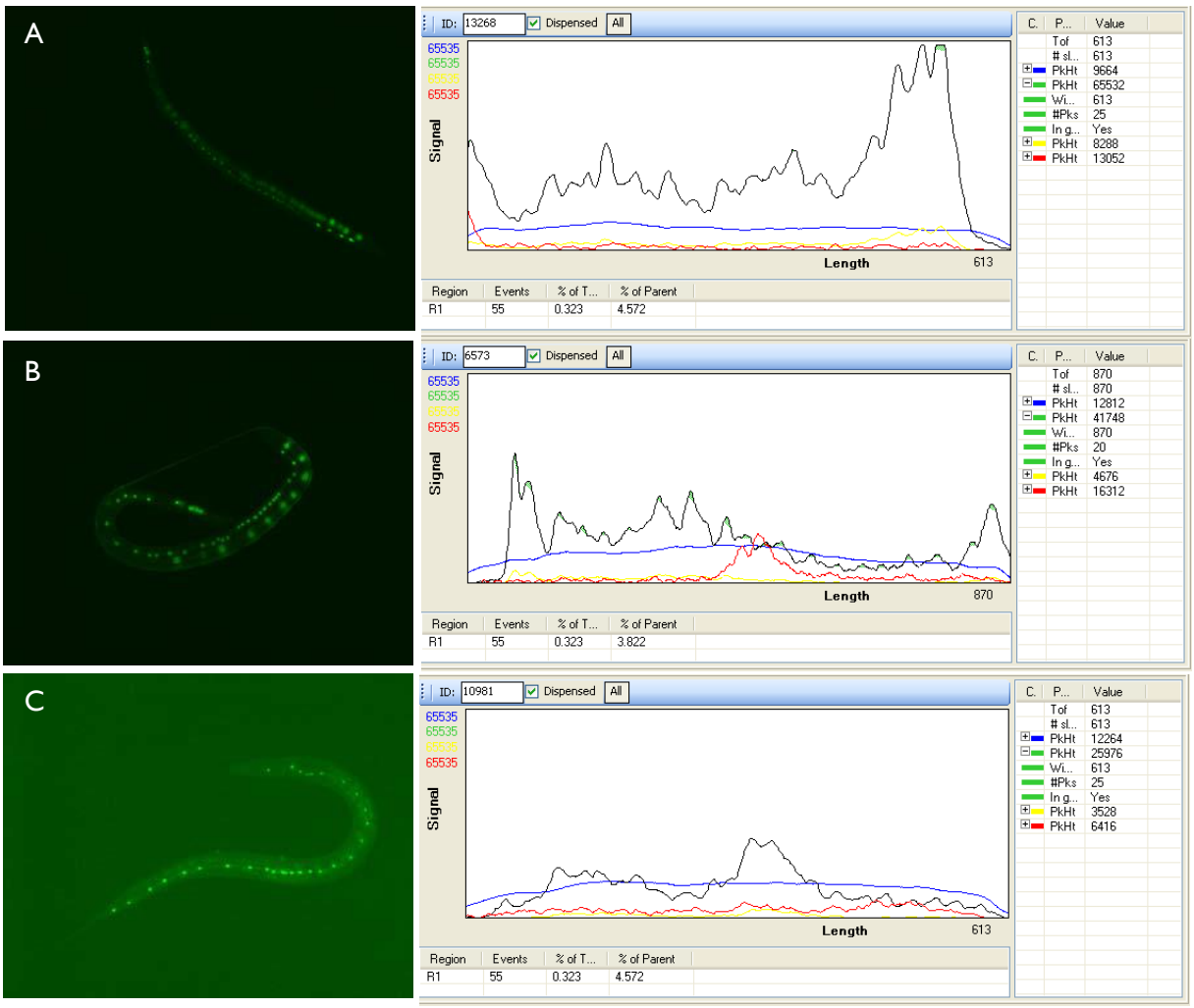


Figure 4a, b and c. *AW 567images and corresponding profiles.*

Average peak count for all worms in the plate of the AW567 was 16.3 (minimum 15, maximum 21).



Average peak count for the worms sorted from the plate containing strain AW568 was 21.6 (minimum 16, maximum 28).

Figure 5a, b and c. *AW 568 images and corresponding profiles.*

Conclusions

Depending on the orientation of the worm as it passes through the flow cell, peaks can be detected correctly. In sample AW567, 26 out of 96 (27% had 16 peaks) worms were counted correctly. Cooling worms for 1 hour in the fridge at 4ºC was necessary to optimize peak counting. Overlapping peaks result in lower peak count numbers. In sample AW568, all of the worms had a count of 16 or more.

The results demonstrate proof of principle that the BioSorter using profiler peak counting should be able to pull out worms with extra seam cells from a mixed population.

Work performed in 2011, QTN reissued 2024:
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