

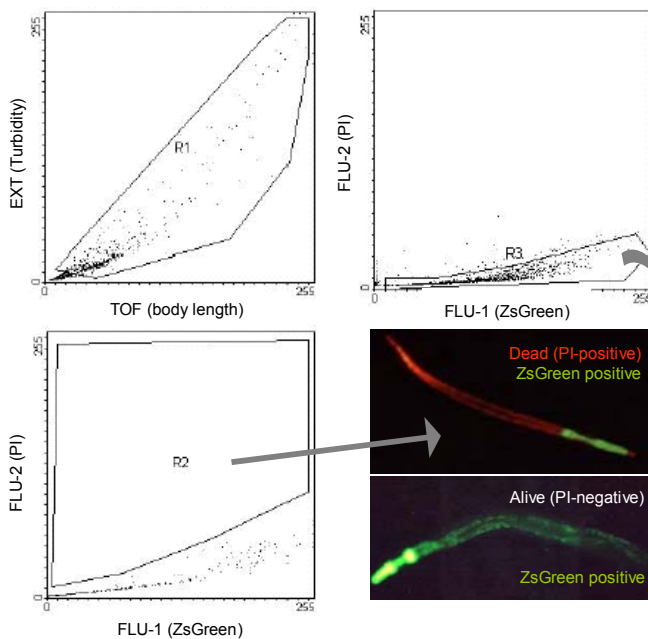
Multi-parametric & Dual Color Fluorescent Analysis and Flow Sorting of *C. elegans*

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Technology. The COPAS BIOSORT is a high speed flow sorter that analyzes and sorts *C. elegans*. The system is equipped with two lasers. A 633 nm excitation diode laser analyzes two physical parameters of the organism: time of flight (TOF, a measure of the length of an organism) and extinction (EXT, a measurement of body opacity). TOF and EXT allow sorting of pure embryos, a larval stage or adults from a wild type population as well as a number of mutants with affected body length or opacity of internal structures.

The 488/514 nm multi-line argon laser is used to measure the expression levels of fluorescent proteins like GFP, YFP, DsRed, ZsGreen, ZsYellow or the binding of fluorophores like SytoxTM-green, propidium iodide (PI) or phycoerythrin (PE)-tagged lectins to *C. elegans* (sub-) populations. Single color applications such as live-from-dead (PI, Sytox green) or male-from-hermaphrodites animals (wheat germ agglutinin-PE) have been developed as well as protocols to isolate animals with predefined levels of expression of a fluorescent protein for e.g. high speed genetic/enhancer/suppressor screens or bulk isolates for micro-array experiments. Here we exemplify how the three photon multiplier tubes (PMT's) of the COPAS BIOSORT allow multi parametric plus dual color sort setting of gatings for TOF/EXT **plus** two fluorescent signals.

Isolation of viable-ZsGreen positive animals.



A mixed *C. elegans* population of wild type (N2) and a strain expressing ZsGreen (Clontech) in the pharyngeal muscle (UBI68) was stained with PI (Molecular Probes) to label dead nematodes. The R1 gating was set to collect all animals and excludes debris. Fluorescent emissions FLU-1 (498-522 nm) corresponds to ZsGreen and FLU-2 (575-595 nm) to PI. The scales of the dot plots are linear. Gates R2 and R3 reflect the 'dead' PI-positive and 'live' & ZsGreen positive populations respectively. In order to evaluate spectral overlap between the filter sets of the two FLU PMT's, negative and single color population

controls are necessary to calibrate instrument settings. Therefore, the purity of sorted PI & ZsGreen 'double positives' (R2) and 'ZsGreen only' populations (R3) were confirmed using microscopy (see micrographs). The results indicated that spectral overlap between ZsGreen and PI is negligible so each population (living versus dead) can be clearly separated from each other in one sort run.

Conclusion. Dual color gatings enable in one COPAS-BIOSORT run, to isolate e.g. a given life stage (TOF) that is positive for a fluorescent protein (FLU-1) from animals that label with the 'dead' marker PI (FLU-2). Multi-parametric dual color applications will facilitate faster bulk isolation of 'pure viable' populations for e.g. micro-array, biochemical or proteomics experiments.