

## **Enhanced Analytical Performance of the *C. elegans* Flow Sorter COPAS BIOSORT: Automated Re-analysis of Populations in Multi-well Plates and Reading of Axially Distributed Positional Fluorescent Signals**

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The COPAS BIOSORT system, used to analyze and sort *C. elegans*, can replace visual inspection and manual picking of nematode populations by accurate, high speed, multi-parametric analysis followed by automated sorting and dispensing in multi-well plates or single recipients. Current COPAS applications include separating egg-to-adult life stages, fluorescent from non-fluorescent animals such as live from dead animals, males from hermaphrodites or those with (altered) expression levels of fluorescent proteins. COPAS also facilitates fast bulk isolation for biochemical or micro-array experiment and automation of genetic/enhancer/suppressor screens. Union Biometrica has developed add-on devices that expand the capacities of COPAS BIOSORT.

***Automated re-analysis of populations in multi-well plates.*** The higher throughput that comes with COPAS in the compound screening lab and the larger numbers of mutants that can be isolated from bigger populations create a new bottleneck at the level of downstream analysis of multi-well plates. For this purpose, the **COPAS ReFLx** has been designed to facilitate re-analysis of populations incubated in 96-well plates. The ReFLx probe will enter the well and while flushing, aspirates the population from the well. While being picked up, the sample is washed and filtered in a ‘bubble trap’ to remove debris of the culture medium and air bubbles that would interfere with the analysis. The population of animals is then rerouted through the flow cell for analysis or for sorting of a desired sub-population. Finally, the animals are wasted or the resorted ones are deposited in the corresponding well of a fresh plate. The ReFLx unit can re-analyze and sort a 96-well plate in less than 45 minutes with a yield of > 90%.

***Reading axially distributed positional information in the *C. elegans* body.*** Inside the COPAS flow cell, the laser beam has been optically focused to a 10 micron section crossing the flow cell, giving rise to fluorescent emission profiles in which details almost the size of a single cell can be resolved axially. To take advantage of this information in the analysis of animal populations and to use it to drive the sorting process, the COPAS Profiler has been developed. It records and stores fluorescent profiles for each animal for analysis purposes. Features in profiles, such as a *predefined* number of ‘peaks’ with *predefined* amplitude and width, can be used as sort criteria. The profiler thus allows the investigator to zoom in from total fluorescence at the organism level, to fluorescent features of organs, tissues, groups of cells and perhaps individual cells in the animal body. A salient feature of axial profiling is that weak fluorescent signals coming from a few cells will be accurately measured when these few cells are axially separated from ‘bulk fluorescence’ coming from major organs. In this way, much more sophisticated forms of analysis and sorting become possible with COPAS technology: as organs, tissues and cell groups are born, dividing, migrating, differentiating or dying in the nematode body.

It is a particularly challenging idea to determine whether with today’s novel fluorescent proteins, the Profiler will be able to accurately discriminate individual cells or perhaps cellular processes that cross multiple optical sections along the A/P axis such as the elongating processes of the excretory canal cell or even axons.