UNION BIOMETRICA
Large Particle Flow Cytometry

COPAS™ XL

Automated Analysis and Sorting of Zebrafish Eggs, Embryos, & Hatchlings

The COPAS™ XL instrument from Union Biometrica, Inc. has been specially designed for the automated analysis, sorting and dispensing of objects up to 1.5mm in diameter on the basis of size, optical density, and fluorescence intensity. COPAS XL is ideal for zebrafish eggs, embryos, and hatchlings. It can also be used for *Drosophila* 3rd instar larvae as well as other similarly sized objects such as large cells / cell clusters, beads and seeds.

Union Biometrica's patented COPAS technology was initially developed for use with *C. elegans* and *D. melanogaster* (fruit fly embryos and larvae). It has now been expanded for use with live zebrafish. The COPAS XL features our largest flow cell yet, as well as modifications to the optical assembly and fluidics to accommodate the larger size of the zebrafish.

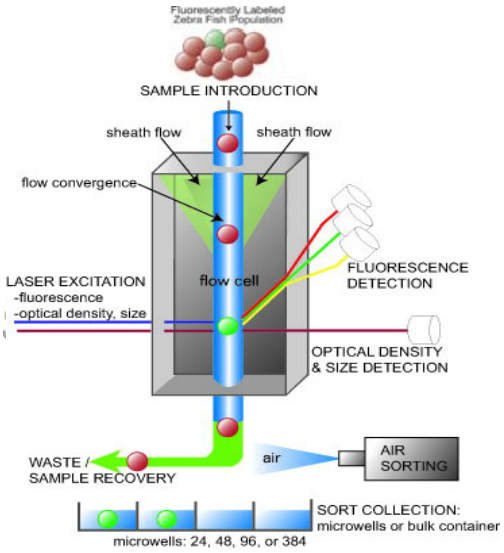
The COPAS (Complex Object Parametric Analysis and Sorting) technology is based on flow cytometry principles; however, it differs from traditional flow cytometers in two key design areas. First, the diameter of the COPAS flow cell can accommodate objects up to 1500 microns, much larger than that of standard flow cytometry instruments used for sorting eukaryotic single cells. The second difference is the patented pneumatic sorting mechanism, which is gentle enough to permit dispensing live animals which are unharmed and ready for follow-on experiments in genetics, toxicology or drug compound screening.

Union Biometrica, Inc. • 84 October Hill Rd, Holliston, MA 01746 USA • Tel: +1-508-893-3115 • Fax: +1-508-893-8044
European Office • Cipalstraat 3 B-2440 , Geel, Belgium • Tel: +32 (0) 14-570628 • Fax: +32 (0) 14-570621
Email: sales@unionbio.com • Internet: http://www.unionbio.com

The Analysis & Sorting Process

The COPAS XL automates the sorting of zebrafish eggs, embryos, and hatchlings by analyzing the animals one by one in a continuously flowing stream. The organisms are passed through the flow cell where five parameters are measured for each organism:

- Optical density (extinction) of the object ("EXT")
- Axial length of the object (size, "TOF")
- Simultaneous detection of up to three colors of fluorescence



Several choices of lasers are available so a system can be optimized to your experimental requirements. Typically the instrument has fluorescence detectors for the green, yellow, and red regions of the spectrum to cover GFP, YFP, DsRed®, and numerous other commercially available fluorophores.

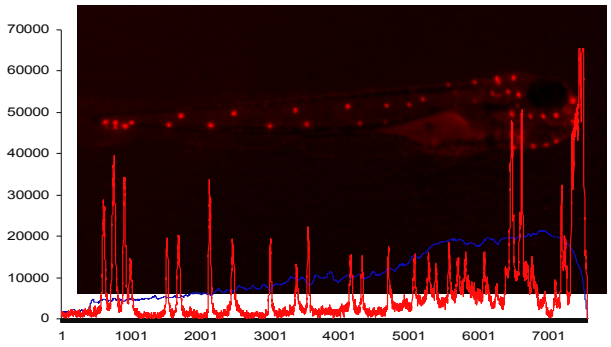
The real-time analysis of these parameters is used to make sort decisions, and only those objects meeting the user-selectable sort criteria are dispensed into microtiter plates or bulk receptacles. Those organisms not meeting the sort criteria are gently sorted by a puff of air to a collection container, where they may be recovered, unharmed and still viable.

Viability

There is no measurable decrease in the viability of live zebrafish after having passed through the COPAS instrument.

Although the sorting rate varies with the concentration of the sample, for zebrafish, it typically takes between 1.5-2 minutes to fill a 96-well microtiter plate with one zebrafish per well. By automating the current time consuming manual processes, this level of throughput permits setting up over 15,000 wells of zebrafish in an assay in an 8-hour day. Research scientists may now more effectively use zebrafish in even large-scale investigations such as in screening campaigns of large drug libraries or genome-wide searches and selection of mutations.

Profiler II Option simultaneously detects and records up to 8,000 data points per object for each of the four channels of extinction and fluorescence. It also includes advanced imaging to graphically and numerically display subtle variations in extinction and fluorescence intensity along the length of an object. Profiler II will digitize the object into a succession of peaks and valleys that directly trace the fluorescence intensity of the object as it passes through the flow cell. The resulting profiles graphically show the location and intensity of all four parameters. Sorting abilities are extended with user-definable sort criteria for profile peak heights, widths, locations, and number for each optical parameter. Profiler II also enables users to optimize their COPAS system by visualizing data, resulting in better detection of strong versus weak signals.



Axial profile (red- fluorescence and extinction) of a stained 4-day old wild type zebra fish larva overlaid with corresponding image.

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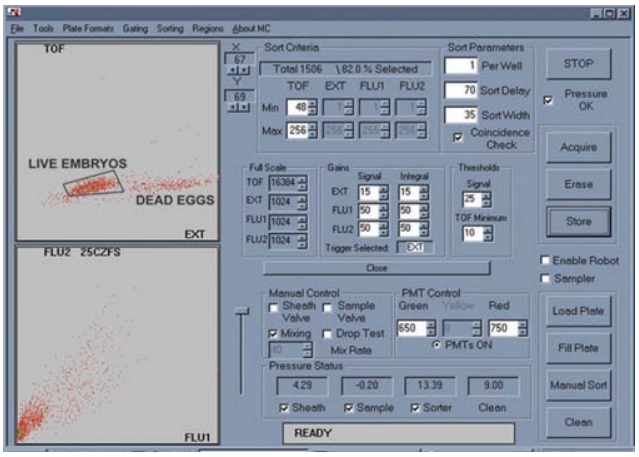
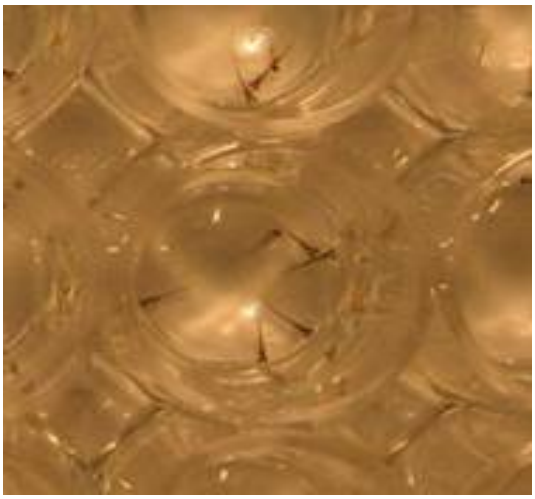
User Interface

The COPAS XL includes an X-Y stage for dispensing into micro-well plates. COPAS software offers the ability to define the numbers of animals dispensed into each well of a 24, 48 or 96-well plate. Selection criteria such as size range, optical density, and/or level of fluorescence intensity are user selectable.

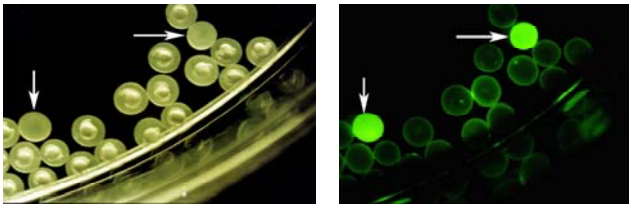
The raw data collected from the analysis is stored both as text, (so that common industry tools may be used for further analysis) and also in a format compatible with most flow cytometry software. The numerical raw data can be easily imported into various analysis programs including common spreadsheet programs to allow for the testing of complex analytical questions and the use of statistics to address subtle biological observations.



Screen capture of COPAS software. Users make sort decisions using this screen by simply drawing a box around the target population.



Six larvae (hatchlings) dispensed per well for an assay.



Images of embryos treated with a live/dead distinguishing stain shown in screen capture above. Arrows highlight dead embryos. Left image under standard microscope; Right image under fluorescence microscope.

Examples of Application Areas

With its two on-board sample cups of 40 ml and 1000 ml, COPAS XL can be used for analyzing and sorting both small and large batches of fish. The COPAS XL includes High Throughput Screening (HTS) features including liquid level sensors, leak detectors, status lights plus automatic sample and pressure control for walk-away operation. In true HTS applications, the system can be interfaced with a robotic plate handler to supply the system with empty plates and remove plates that have been filled. Sample customer applications include:

- Automated dispensing of organisms into microtiter format for rapid assay preparation
- Population enrichment prior to further experiments, such as the separation of dead organisms from live.
- Quantification of the level of fluorescence from analyzed zebrafish, which may be auto-fluorescence, fluorescent protein expression, or fluorescent binding markers.
- Isolation of rare viable mutants using measurable physical parameters

System Specifications COPAS™ XL

Object Size (Diameter) Range	100 – 1500 micron
Object Parameters Measured	The system is capable of quickly analyzing both small and large quantities of objects using 5 parameters: size, optical density and 3 channels of fluorescence.
Fluorescence (FLU)	3 color fluorescence: The fluorescence intensity of three different wavelengths can be simultaneously determined by the excitation and emission filters in the system.
Size (TOF)	Relative size (the object's axial length) is referred to as the object's time of flight (TOF). It is determined by the time that the light blockage signal remains above a pre-set threshold level.
Optical Density (EXT)	The optical density (how dark or transparent an object appears) is referred to as the object's extinction (EXT). It is determined by the total integrated signal of the light blockage.
Collection devices	Bulk receptacle such as a Petri dish 24, 48, and 96-well plates
Drop Size	Approximately 40 µl
Laser Excitation Wavelengths	System accommodates two lasers. Inquire for details on the latest combinations of lasers available. Choices include: • 488/514 nm multiline argon-ion gas laser for fluorescence used with 670 or 635 nm laser diode for EXT & TOF. • 405, 488 & 561 nm solid state lasers
Detectors	One PIN Photodiode for measuring forward scatter (EXT) and time of flight (TOF) 3 photomultiplier tubes for measuring Green, Yellow, and Red fluorescence.
Sample Cup Capacity	Two sample containers: 1 x 1,000 ml and 1 x 40 ml
Sample Mixing	Magnetic stirrer bar for mixing in sample cups
Sorter Mechanism	Gentle air jet fluid diverter activated from signal processing electronics
Sample Viability	There is no noticeable decrease in the viability of live embryos after having passed through the COPAS instrument.
Speed (analysis & counting)	Maximum 20 objects / sec (based on maximum sample concentration and nominal sample flow rates with coincidence check enabled)
Sort Purity	>98%
Fill Time for 96-well Microtiter Plate	1.5-2 minutes filling time on average per 96-well plate with 1 organism per well selected, coincidence check software operating, nominal sample concentrations, and an acquisition rate of 1-5 organisms per second in a selected region
Dispensing Accuracy	>95% of wells filled have one organism. Of the filled wells, <2% may have 2 or more organisms
System Weight	COPAS instrument: 88 lbs (40 kg) not including external laser or PC
Instrument Dimensions	2 ft (0.6 m) deep x 20 inches (0.5 m) wide
Computer	IBM compatible PC with color monitor

For more details, applications information, pricing, and availability, please contact Union Biometrica, Inc.

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