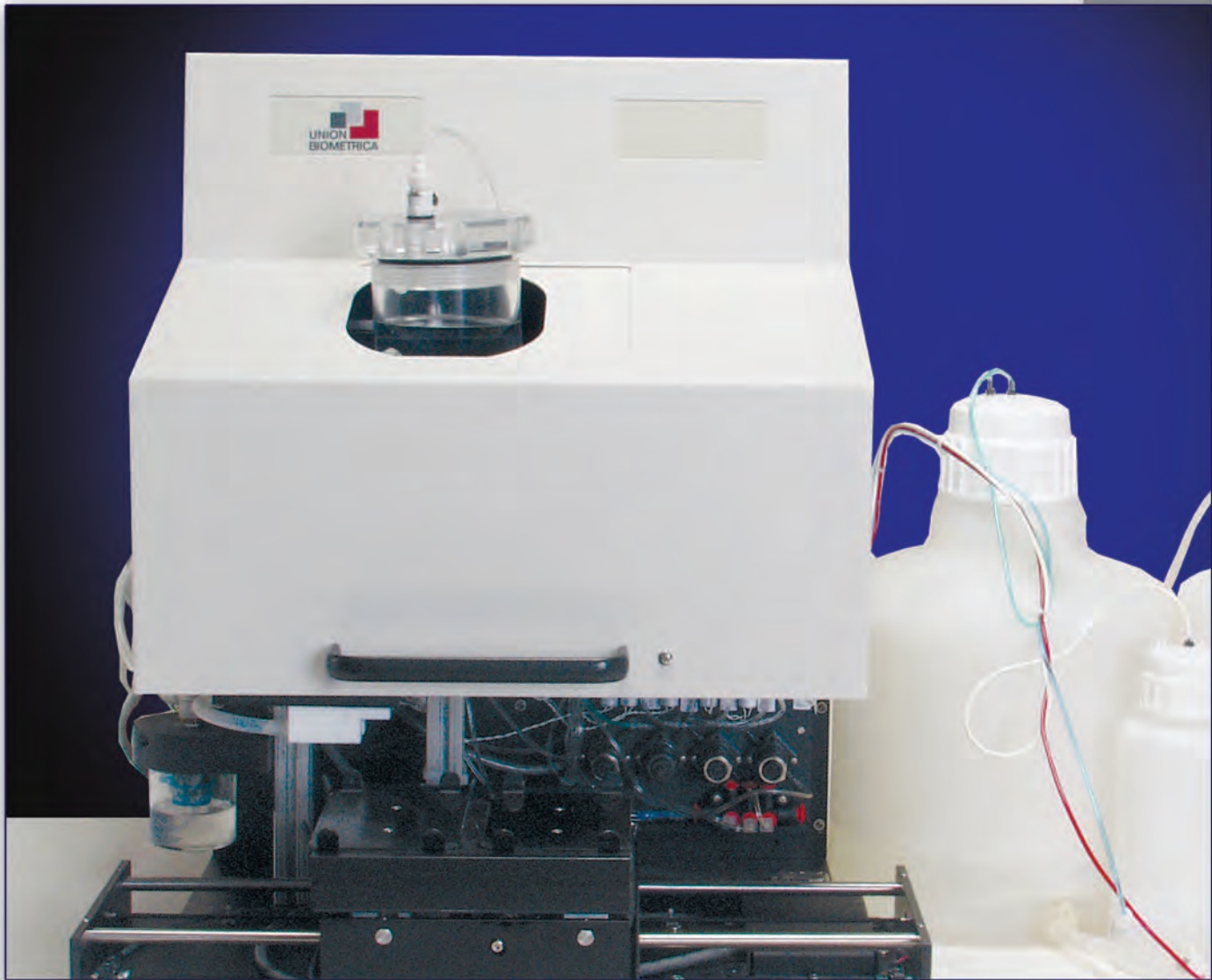


COPAS™ Instruments for Large Particle Flow Cytometry

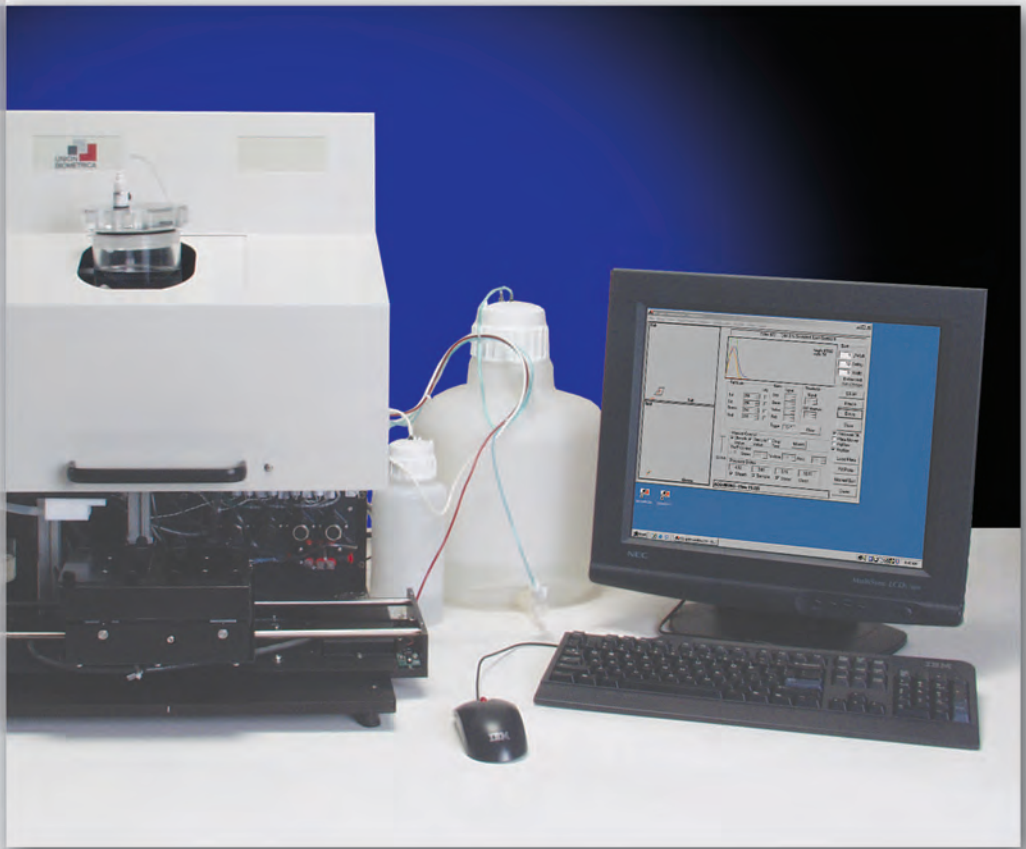


Flow sorting systems for the automated analysis and sorting of viable multicellular organisms, cells, and other objects sized from 20-1500 microns.

Established in 1998,

Union Biometrica is the pioneer in flow cytometry systems for high-throughput analysis, sorting, and dispensing of objects ranging in size from approximately 20 to 1500 microns. Originally designed for the analysis and sorting of small multicellular organisms, (primarily the nematode, *C. elegans*, and *Drosophila* embryos and larvae), our COPAS™ (Complex Object Parametric Analyzer and Sorter) instruments made automated screening possible for these research areas and rapidly became known as the “worm” and/or “embryo” sorters in scientific communities. As our reputation grew, researchers from other application areas began requesting instruments for sorting larger objects, such as *Arabidopsis* seeds and zebrafish embryos. In 2002 we released our largest platform instrument with a 2 mm flow cell. Today, Union Biometrica offers systems in a variety of size-optimized platforms that are capable of sorting objects by length, optical density, and multiple channels of fluorescence.

Through partnerships with leading medical and research institutes, we have developed our second generation of COPAS™ instruments and software. These have expanded the instruments' applications into cell areas such as adipocytes (fat cells), hepatocytes, oocytes, pancreatic islets, and embryonic stem cells and cell clusters. Union Biometrica remains committed to continuous development of unique, innovative solutions and bringing automation to an expanding range of research areas that have been considered too large for traditional flow sorting and analysis technologies. We also offer a specially formulated line of reagents and control particles.



The “big” difference...

Union Biometrica's COPAS™ large partical flow cytometers have been specially designed for the analysis, sorting, and dispensing of objects from 20 to 1500 µm. Based on the same principles as traditional flow cytometry, our instruments offer researchers increased speed, sensitivity, quantification, and repeatability in areas of research previously limited to manual analysis and sorting. Our COPAS™ instruments differ from traditional flow cytometers in two important design areas (picture shown below) to permit larger objects to be analyzed and dispensed:

1. The **large-bore fluidics and flow cell** can accommodate much larger objects than that of traditional flow cytometry instruments. Each of the four system platforms features a specially engineered fluidic path and flow cell optimized for specific object size ranges to achieve the highest measurement accuracy and sensitivity at the maximum speed and throughput possible.
2. The **patented pneumatic sorting mechanism**, first introduced in our proven COPAS instruments, sorts objects with a gentle puff of air. This non-destructive sorting method permits the analysis and collection of live organisms, cells, tissues, or sensitive chemistries with a high level of recovery and a low level of lethality. Traditional flow cytometers use electrostatic sorting methods that are potentially damaging to live objects.

Five physical parameters are optically measured and recorded for each object of interest:

- Optical density (extinction)
- Axial length (time of flight)
- Fluorescence emissions, simultaneously by three different detectors

Application Areas Include

Large Cells/Cell Clusters

- Adipocytes
- Duct Cells (Kidney, Pancreatic, etc.)
- Embryoid Bodies
- Hepatocytes
- Pancreatic Islets

Beads & Particles

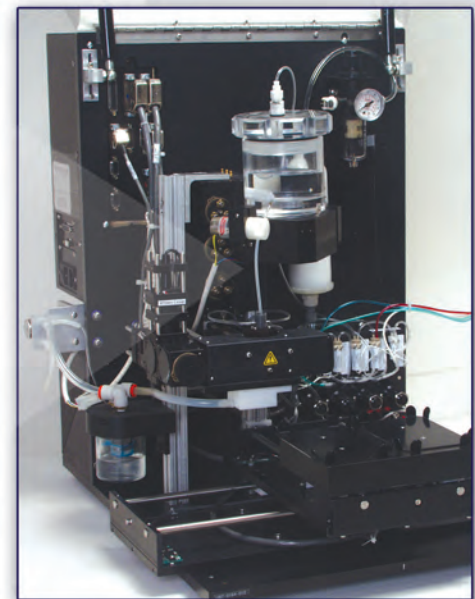
- Combinatorial Chemistry Beads
- Bead Based Assays

Small Multicellular Organisms

- *C. elegans*
- *D. melanogaster*
- Zebrafish
- Medaka
- Mosquito
- *Xenopus*
- *Daphnia*

Small Plant Models

- *Arabidopsis* seeds
- Pollen

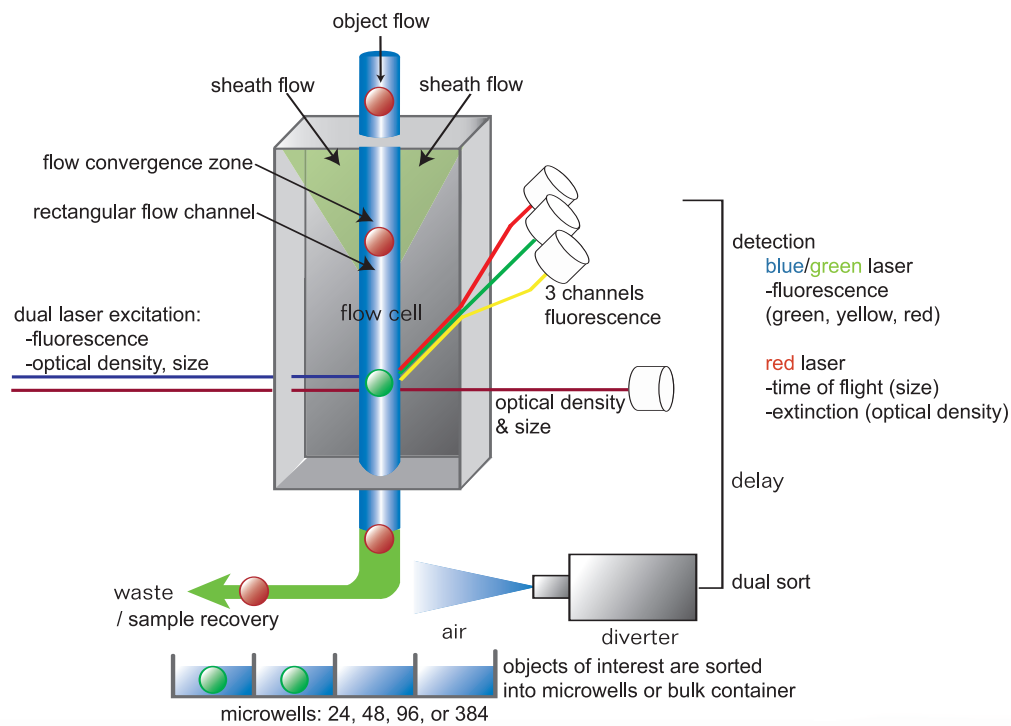


Sorting out the process...

Objects flow from a continuously-mixed sample cup to a pre-analysis chamber, where the sample is surrounded by a "sheath" solution to produce laminar flow and focus the objects in the center of the flow stream. The objects then pass into the flow cell where they are illuminated by two low energy lasers. A red diode laser (670 nm) is used to measure the axial length and the optical density of the object, and a multi-line argon laser (488 / 514 nm) is used to excite fluorescence. In the standard configuration, the instrument has fluorescence detectors for the green, yellow, and red regions of the visible spectrum. The system can also be configured with multiple optical filters and a variety of alternate laser configurations (including UV) to create a customized solution to meet specific applications requirements.

Real-time analysis of these measured parameters is used to make sort decisions using our proprietary software. Only those objects meeting user-set sort criteria are dispensed, unharmed and viable, into microtiter plates or bulk collection receptacles. Objects not meeting the sort criteria are gently directed by our patented pneumatic sorting mechanism to a sample container where they also may be recovered.

Diagram of the object flow path and sorting technique



Analyzing the details...

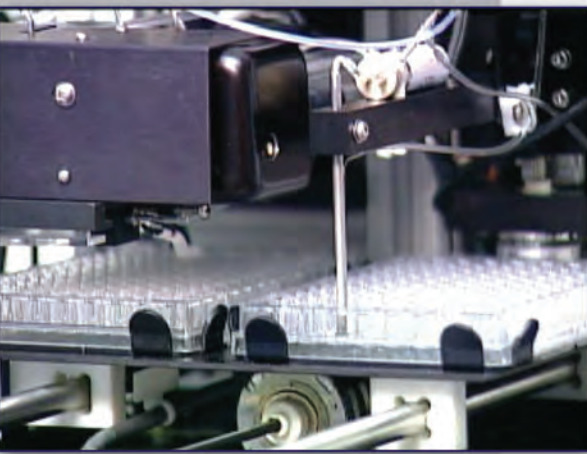
Researchers may create, store, and retrieve specific assay files and experimental results. Raw data collected from the analysis is stored both as a text file and in the List Mode Data (LMD) format which is compatible with most flow cytometry analysis software. The numerical raw data can also be easily imported into various analysis programs, including commonly used spreadsheet programs, to allow for the testing of complex analytical questions and the use of statistics to address subtle biological observations as well as strengthen conclusions from the numerical data.



Proprietary software provides a user-friendly interface for viewing data and changing experiment settings.

Sizing up your instrument needs...

ReFLx Sampler option
for object re-analysis

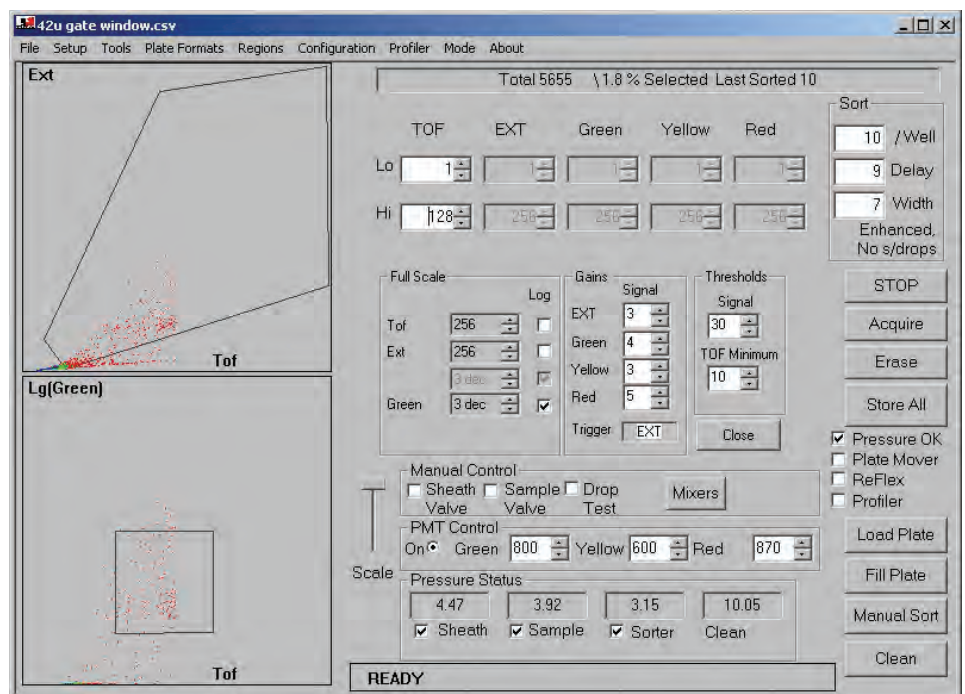


Union Biometrica works with our customers to configure the right technology platform to meet your individual lab requirements. Instruments range from bulk dispensers to fully automated systems with user-selectable optical filters, lasers, hardware and software enhancements. Instruments can also be interfaced with robotic plate handling devices for high-throughput applications.

ReFLx Sampler is an integrated sample injection system designed for follow-up analysis/re-analysis of objects from a 96-well microtiter plate. The sampler gently agitates and aspirates samples from a 96-well plate into the instrument's flow cell for analysis. The analyzed samples can then be dispensed into the corresponding wells of a new 96-well plate according to user-set criteria. Includes both hardware and software additions to the COPAS™ systems. Not available on all platforms.

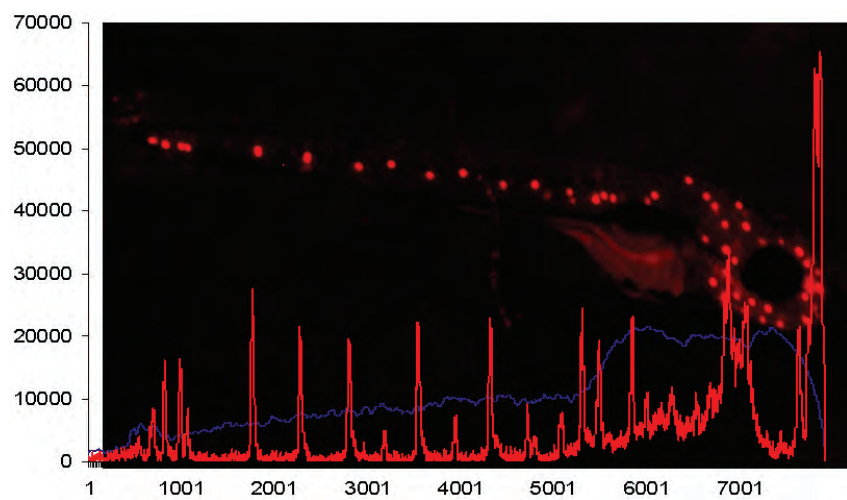
Advanced Acquisition Package includes advanced electronics and enhanced software analytical tools that permit significantly greater data resolution, object flow, and acquisition rates. Higher signal resolution is achieved with 16-bit data resolution (1:65536) to offer 32 times more detail in intensity and size than our standard software, and the ability to detect smaller objects, distinguish better than 5 μm size differences, and sense lower intensity fluorescent signals. The increased object acquisition rate permits the sample to be run four times faster than standard rates. Additional software features include: improved data displays including linear and logarithmic scaling; extended sorting capabilities; fluorescence compensation for increased fluorescence discrimination; user-defined mathematical functions for advanced real-time data manipulation; and expanded data storage capabilities fully compatible with FCS 2.0 standard. Includes both hardware and software additions to the COPAS™ systems.

Advanced Acquisition Package software in dot-plot mode showing size (Tof), density (Ext), and fluorescence (LgGreen) data for a mixed-stage population of *C. elegans* expressing green fluorescence protein. Each dot represents one worm.



Profiler II (includes Advanced Acquisition Package) simultaneously detects and records up to 8,000 data points per object for each of the four optical parameters - extinction and three fluorescence channels. Includes advanced imaging to graphically and numerically display subtle variations in fluorescence and extinction intensity along the length of an object. While the standard software provides a single integrated signal measurement

for each parameter of an object, the 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 result is an optical profile of each object graphically showing the location and intensity of all four optical 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 COPAS™ systems by visualizing data, resulting in better detection of strong versus weak signals. Includes both hardware and software additions to COPAS™ systems.



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

	BIOSORT	SELECT	PLUS	XL
Flow Cell Size	250 microns	500 microns	1000 microns	2000 microns
Object Size Range	~40-200 microns	~40-350 microns	~40-700 microns	NA
Object Size Range w/ AAP	~20-200 microns	~20-350 microns	~30-700 microns	~100-1500 microns
Analysis & Counting Rate	35 events per second (eps)	25 eps	15 eps	NA
Analysis & Counting Rate w/ AAP	150 eps	100 eps	100 eps	20 eps
Dispensing Rate into Stationary Bulk Receptacle	15 eps	10 eps	5 eps	NA
Dispensing Rate into Stationary Bulk Receptacle w/ AAP	25 eps	20 eps	10 eps	2 eps
Dispensing Fill Time for 96-well plate (1 object / well)	Minimum 1.5 – 2 minutes per plate	Minimum 1.5 – 2 minutes per plate	Minimum 1.5 – 2 minutes per plate	Minimum 1.5 – 2 minutes per plate
Dispensing Fill Time for 384-well plate	Minimum 2 – 3 minutes per plate (20/well)	Minimum 2 – 3 minutes per plate (10/well)	Minimum 2 – 3 minutes per plate (5/well)	Minimum 2 – 3 minutes per plate (2/well)
Automated Dispensing Accuracy (1/well)	Greater than 98% of wells filled have one or more objects. Of the filled wells, less than 5% may have 2 or more objects.	Greater than 98% of wells filled have one or more objects. Of the filled wells, less than 5% may have 2 or more objects.	Greater than 98% of wells filled have one or more objects. Of the filled wells, less than 5% may have 2 or more objects.	Greater than 95% of wells filled have one or more objects. Of the filled wells, less than 5% may have 2 or more objects.
Throughput (per well) (w/ one stationary bulk receptacle)	up to 570/well	up to 400/well	up to 40/well	up to 10/well
Collection types	24, 48, 96 or 384 well plates. Stationary bulk receptacle.	24, 48 or 96 well plates. Stationary bulk receptacle.	24, 48 or 96 well plates. Stationary bulk receptacle.	24, 48 or 96 well plates. Stationary bulk receptacle.
Excitation Laser options	488/514 nm multiline Argon-ion with 670 (or 635) nm diode. 405, 488 and 561 nm solid state lasers. UV325 nm and 375 nm lasers.	488/514 nm multiline Argon-ion with 670 (or 635) nm diode. 405, 488 and 561 nm solid state lasers. UV325 nm and 375 nm lasers.	488/514 nm multiline Argon-ion with 670 (or 635) nm diode. 405, 488 and 561 nm solid state lasers. UV325 nm and 375 nm lasers.	488/514 nm multiline Argon-ion with 670 or 635 nm diode. 405, 488, 561 or 405 nm solid state lasers.
Detection Optics	Extinction + 3 PMTs (Green, Yellow, Red). Others available.	Extinction + 3 PMTs (Green, Yellow, Red). Others available.	Extinction + 3 PMTs (Green, Yellow, Red). Others available.	Extinction + 3 PMTs (Green, Yellow, Red). Others available.
Instrument Footprint	0.5m(W) x 0.6m(D)	0.5m(W) x 0.6m(D)	0.5m(W) x 0.6m(D)	0.5m(W) x 0.6m(D)
Recommended work space	2.0m(W) x 0.6m(D)	2.0m(W) x 0.6m(D)	2.0m(W) x 0.6m(D)	2.0m(W) x 0.6m(D)
Weight	40kg	40kg	40kg	40kg

(AAP: Advanced Acquisition Package significantly increases instrument capabilities, and has been noted separately.)

For research purposes only. Not for use in therapeutic or diagnostic procedures.

U.S. patent nos 6,657,713, December 2, 2003 & 6,400,453, June 4, 2002, Large Object Sorter: Fluid Controlled Machine for Selecting and Depositing Multicellular Organisms

U.S. patent no. 7,116,407, October 3, 2006, System For Axial Pattern Analysis of Multicellular Organisms (Profiler)

Canadian patent no 2,341,231, October 21, 2003, Large Object Sorter: Fluid Controlled Machine for Selecting and Depositing Multicellular Organisms

COPAS™ is a trademark of Union Biometrica, Inc.

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