COPAS *Infinity*™

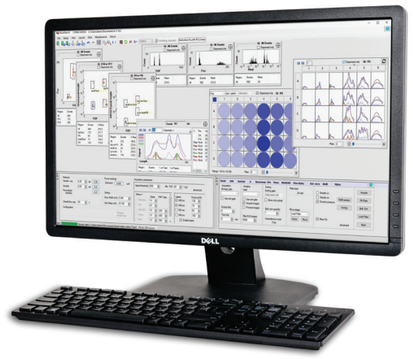
Large Particle Flow Cytometer

Flow sorting systems for automated analysis and sorting of viable cells, cell clusters, small multicellular organisms and other 2-1500 micron-sized objects.

UNION BIOMETRICA

Large Particle Analysis and Sorting

Large Particle Flow Cytometry



Traditional flow cytometry is well established for analyzing, and in some cases sorting, single cells. Historically this technology was not available for the study of larger objects, such as larger cells, cell clusters, and small multicellular organisms. Researchers of these larger sample types were limited to the use of microscopes for analysis and manual manipulation, techniques that are tedious, error prone, and severely limited in throughput. So there was a need for high throughput sorting technology of larger objects.

The COPAS family of sorters provide flow cytometry instruments that overcome these challenges. As with our original COPAS platform of flow cytometers the **COPAS Infinity with FlowPilot software** provides automated high throughput analysis and sorting of viable multicellular organisms, cell clusters, single cells, bead-based libraries, and particle collections that are too large or too fragile for traditional flow cytometers. Additionally, **COPAS Infinity** has been designed with increased sensitivity at the smaller size range and expanded number of detectors for measurements, all together in a smaller footprint.

There are benefits to studying multicellular structures intact rather than reducing them to their individual cell components. Once cells self-organize into clusters they communicate and behave differently than in isolation. The **COPAS Infinity** large particle cytometer allows you to study the cell-cell interactions found in tissues, tumors, or organoids without the need to disrupt the clusters as for traditional analysis. Or, if you are working with model organisms, replacing manual sorting with a **COPAS Infinity** instrument provides fast, sensitive, reproducible automation for gentle sorting and high throughput screens.

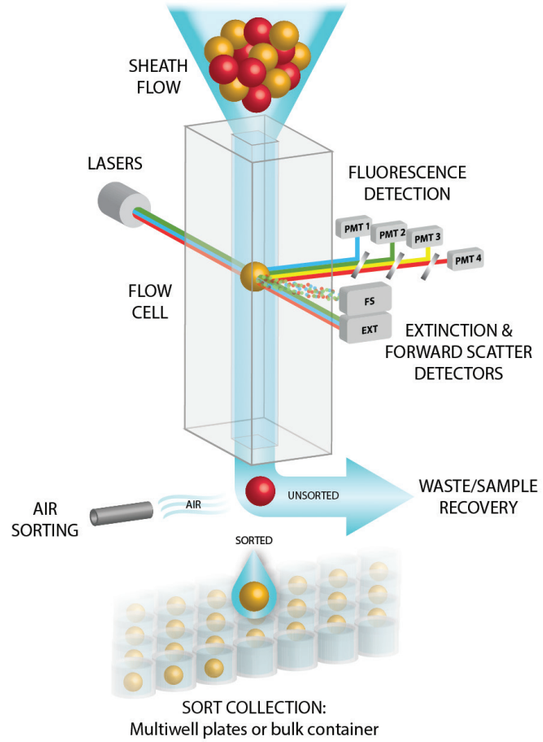
Each of the four **COPAS Infinity** models features an optimized fluidic path, flow cell and optics engineered for a specific subset of the size range delivering maximum sensitive, speed, accuracy and throughput. Instruments can dispense objects either into bulk receptacles or multiwell plates. Each **COPAS Infinity** system can be equipped with up to three lasers. Six optical detectors measure up to 31 different fundamental parameters per each object in a sample. PIN diode detectors measure size (TOF), optical density (EXT) and forward scattered light (FS). Four PMT detectors measure side scatter and fluorescence emissions. All this is combined in an instrument that fits conveniently in a biosafety cabinet.

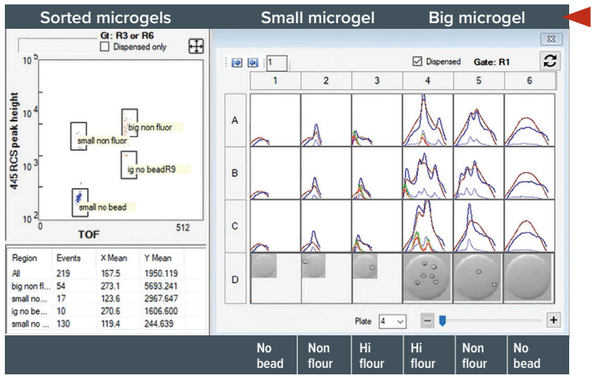
Patented, Gentle Sorting Mechanism

Samples travel from a continuously stirred sample cup to a flow cell where they are carried by a sheath solution which hydro-dynamically focuses them into the center of the stream for interrogation by up to three lasers. The combined fluid stream exiting the flow channel is continuously diverted by an air stream to a waste/recovery container until a “sort” signal is produced. If an interrogated object’s measurements meet the sort criteria, the air diverter is briefly turned off to generate a droplet of fluid containing the sort-selected object which then falls directly below the exit of the nozzle into a collection vessel of the operator’s choosing.

The COPAS platform of instruments utilizes the same fundamental principles of flow cytometry but differs from traditional flow cytometers in several important design areas:

- First, the large-bore fluidics of the COPAS instruments can accommodate objects from 2 to 1500 microns wide, a range that is much larger than traditional flow cytometers.
- Second, COPAS systems operate at slower flow rates and lower pressures thereby avoiding the potentially disruptive high shear forces inherent in standard flow cytometers.
- A third difference, is at the heart of our COPAS technology. The patented pneumatic sorting mechanism, located downstream of the flow cell, utilizes an air diverter to dispense organisms and large cells in a fluid drop. Comparatively, traditional cytometers typically rely on mechanical sorting or application of a large electrostatic charge. Both of these have limitations when large particle samples are involved.





Selecting gate regions for dispensing. Microscope captured images verify the nature of the sorted microgel particles.

The instrument operator can select to sort all particles in a sample or define a gate region to sort only a subfraction of the entire population, only those that meet certain sort criteria. An example of gated regions and the resulting sort on a COPAS system is shown on the left. The sample shown is a mixture of two sizes of microgel spheres, small microgel and big microgel, either encapsulating one or more fluorescent beads or lacking any of these fluorescent beads. In this example four regions are defined by the operator on the TOF (size measurement) vs fluorescent peak height dot plot. COPAS dispenses particles matching one criterion at a time. Single microgel spheres were dispensed to wells of a multiwell plate, first from the “small no bead” region, then subsequently from the other three regions. The Profiler data of each microgel sphere is displayed. The Profiler data show the specific pattern and regional distribution of fluorescence expression within the microgel. Representative images from the sorted regions area are shown below the profiles.

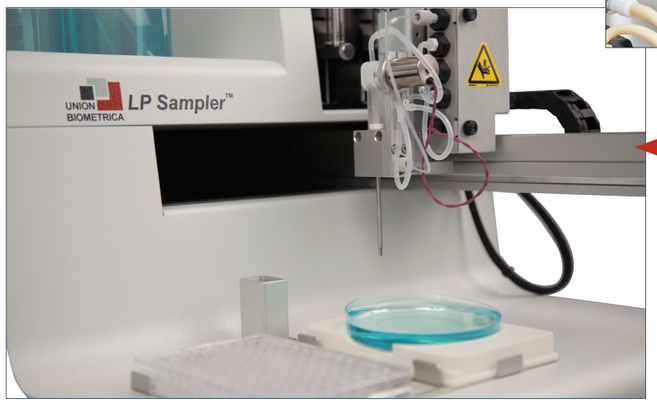
Sample Introduction

Sample introduction in the base configuration is from a 50 ml conical tube (40 ml working volume) with suspended stirring mechanism.

Optional 750 and 1500 ml stirred sample cups are available for larger volume samples as needed in larger screens.

The **Oscillating Sample Introduction System (OSIS)** is designed for handling delicate samples, like adipocytes, skeletal muscle fibers, plant protoplasts, and others, especially ones that either float or sink with traditional stirring agitation. OSIS gently agitates samples without direct contact of a mechanical stirrer.

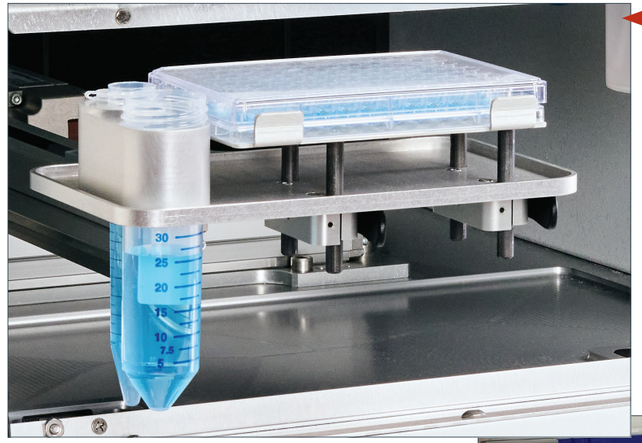
Stirred sample introduction cups



COPAS Infinity™

Sample Types

The **Large Particle (LP) Sampler**™ can introduce the full range of sample types from wells of multiwell plates, Petri dishes, microfuge tubes, and other similar sample containers.



Dispensing stage for sorting

Sample Output

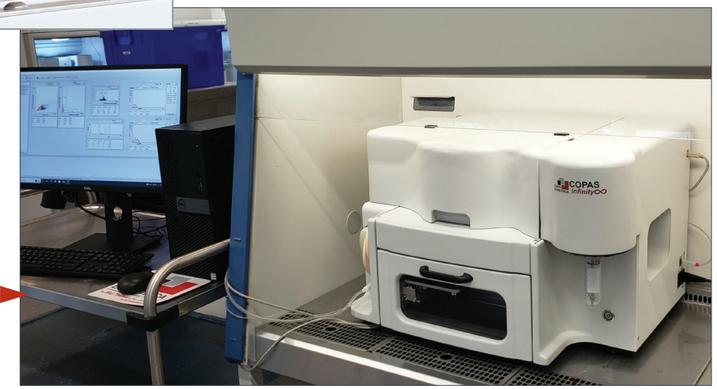
The X-Y stage allows dispensing into 24-, 48-, 96-, or 384-well multiwell plates, tubes, and bulk receptacles. **Dispensing occurs within an enclosed chamber**, providing aerosol containment and UV sterilization to enhance biosafety and sterility.

With all COPAS instruments dispensing is not limited to standard multiwell plates. The user can create custom output receptacle templates. Each dispense location may be given a different combination of sort conditions, such as number of objects and gate region.

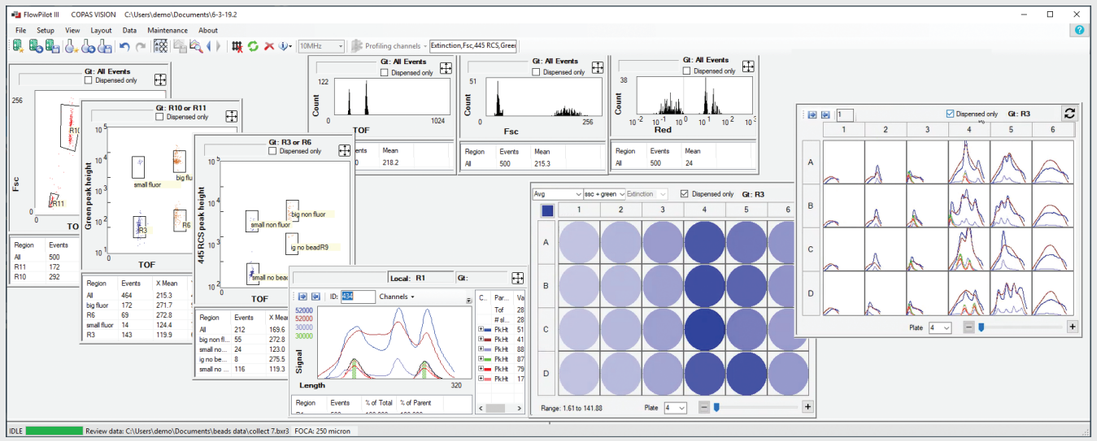
Integration

The COPAS Infinity can be integrated as one component of a multi-step workflow process. Software and hardware connections allow COPAS Infinity to respond to command signals from an outside controller and scheduling software.

The COPAS Infinity was designed with a small footprint that enables it to more easily fit inside commercially available biosafety cabinets, providing both increased safety and sterility.



COPAS Infinity™ fits comfortably in a biosafety cabinet



FlowPilot™ Software for System Control & Data Analysis

Union Biometrica's FlowPilot software was developed for the COPAS platform of instruments with the demanding flow cytometry user in mind. Intuitive and easy-to-use, you don't have to be an expert to begin using FlowPilot equipped instruments.

The dynamic FlowPilot desktop allows the user to easily access or hide instrument control, data acquisition, and dispensing panels based on personal preference. Users can define and manipulate multiple independent graphical and statistical displays of acquired data including multiple regions per plot, custom scaling and logical gating options. Retrievable experiment and sample template files as well as options included for data review (on-instrument or off-line) provide powerful tools for post-acquisition analysis. The user can create custom output receptacle templates for dispensing with well-to well dimensions as dense as 384 well standards. Data is also stored in standard flow cytometry format so it can be analyzed later with other flow cytometry software that may be available in your laboratory.

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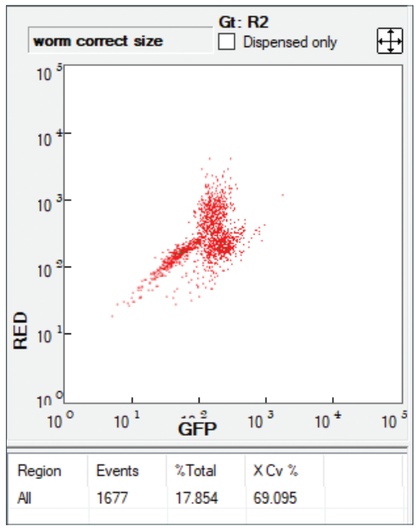
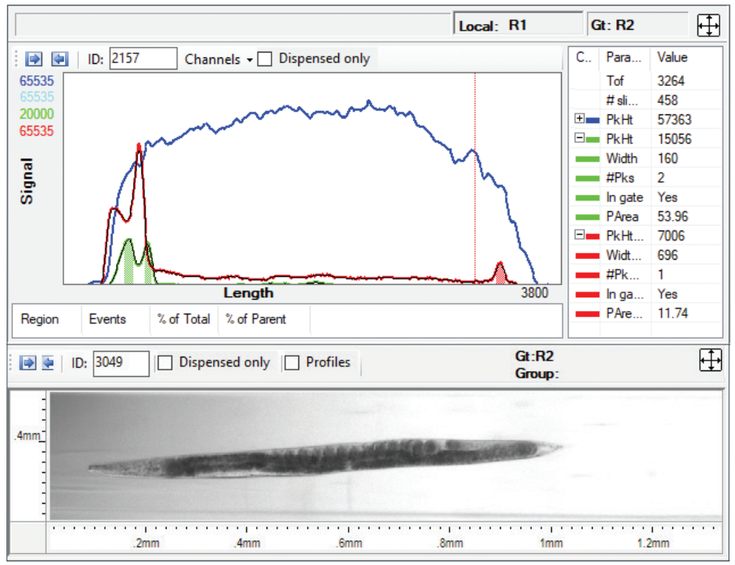


Profiler Feature

Profiler takes data collection to the next level by simultaneously recording the intensities of extinction, forward scattter, and fluorescence along the length of each object. Unique to Union Biometrica instruments, the Profiler feature digitizes objects at rates up to 10 MHz allowing for detection of micron-sized features within the object. The software graphically displays these optical parameters as a succession of peaks and valleys that directly trace the detected signal intensities internal to the object as it traveled through the flow cell. Each object's resulting profile graphically show the location and intensity of all optical parameters collected. Analysis capabilities are expanded with user definable profile criteria using peak heights, widths, integral values, locations and number of peaks. Additional parameter manipulation can be employed to produce ratiometric analyses – all of which can be used as sort criteria.

Another profiling feature is **Partial Profiling**. By focusing in on one region of the profile, Partial Profiling allows the user to strategically identify optical or fluorescence characteristics from that area alone. With Partial Profiling active, profile features (peak height, width, or count) as well as integrated values over that limited portion are now analyzed and graphed as their own customized parameter. Partial Profiling can be configured to analyze extinction, forward scatter, and fluorescence measurements exclusively to selected regions of the ends or middle of the sample particle being analyzed.

The example at the right shows identification of red fluorescence in neurons in the tail of a *C. elegans* animal expressing red fluorescent marker in these neurons, even though red expression exists more strongly elsewhere in the animal. (Profile graph highlights green peak area in the head and red peak area in the tail: profile summary lists characteristics identified in each profiled channel). Representative brightfield image of an adult *C. elegans* animal with the displayed profile.



Partial Profiling of C.elegans adult

Data Review

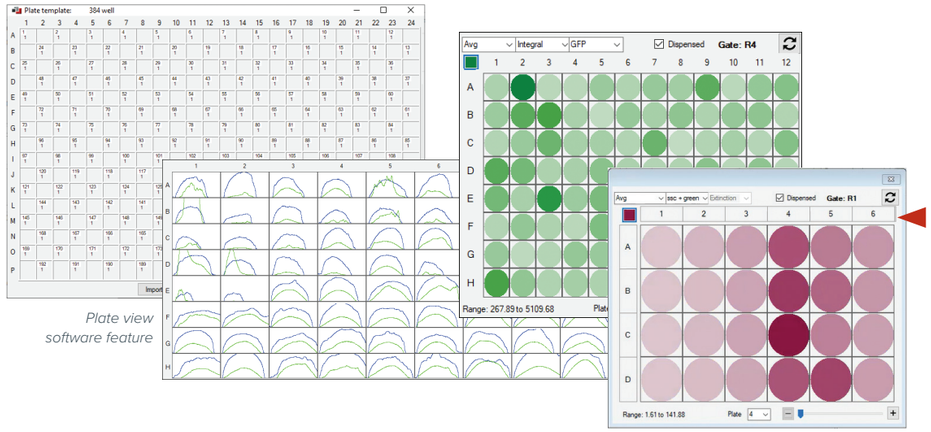


Plate view software feature

Plate view feature allows presentation of data according to the plate template used during acquisition or dispensing.

Summary Of Available Options

Choice of a Flow Cell: 250, 500, 1000, 2000 µm

Choose the appropriate flow cell to process sample type. *(Examples are in the table below. Note these are general guidelines. Please talk to one of our Application Scientist about your specific project and sample requirements.)*

	Recommended Object Size	Typical Examples
250 µm Flow Cell	10–175 µm	*C. elegans* iPSCs
500 µm Flow Cell	30–350 µm	Drosophila embryos Plant protoplasts Mammalian adipocytes
1000 µm Flow Cell	30–750 µm	Spheroids Organoids
2000 µm Flow Cell	40–1500 µm	Zebrafish embryos Plant calli

Multi-laser Configurations

Up to three (3) lasers can be configured depending on the particular fluorophores to be analyzed.

Sample Introduction

Choose stirred sample cups of 50, 750 or 1500 ml sizes. The **OSIS chamber** can be used for extremely delicate samples or samples that float or sink.

Large Particle (LP) Sampler™

Large Particle (LP) Sampler

This sample introduction system is designed to remove samples from wells of multiwell plates, Petri dishes, microfuge tubes, and other similar sample containers and transfer the samples to the COPAS Infinity system for analysis and dispensing.

Integration Feature

The **COPAS Infinity** can be integrated as one component of a multi-step workflow process. Software and hardware connections allow **COPAS Infinity** to respond to command signals from an outside controller and scheduling software.

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Large Particle Flow Cytometer

COPAS Infinity™ with LP Sampler

Examples of Application Areas

For more details you can see 200+ customer journal publications and posters at unionbio.com/publications.

Large Cells/ Cell Clusters	Beads & Particles	Small Multi-Cellular Model Animals	Small Plant Models
• Adipocytes • Cardiomyocytes • Duct Cells (kidney, pancreatic, etc.) • Pancreatic Islets • Stem Cell Clusters / EBs • Spheroids & Organoids (mammary, neurospheres, intestinal, tumorspheres)	• Bead Based Assays • Cells in & on beads • Encapsulated Samples • Microspheres	• *C. elegans* • *D. melanogaster* • Marine Plankton • *Medaka* • Mosquito • Zebrafish (*D. rerio*)	• *Arabidopsis* & *Nicotiana* Seeds • Calli • Fungi • Pollen • Protoplasts

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