COPAS *VISION* ™

Large Particle Flow Cytometer With Image Capture

Flow sorting systems for the automated analysis, dispensing and brightfield image capture of viable multicellular organisms, cell clusters, bead-like particles and other sample types that are too large or too fragile for traditional flow cytometers.

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Large Particle Analysis and Sorting

Large Particle Flow Cytometry

Traditional flow cytometry is well established for analyzing, and in some cases, sorting single cells. But researchers studying larger objects were historically limited to manual manipulation with a microscope – a technique that is tedious, error prone and severely limits throughput. This identified a need for high-throughput sorting technologies for larger objects.

Since 1998 researchers have been using Union Biometrica's COPAS™ family of sorters for **analysis and sorting of particles which are too large or too fragile for traditional flow cytometers.** These systems operate at lower pressures and use a proprietary, gentle air stream diverter for sorting. COPAS instruments come with a single fixed flow cell selected for optimal use depending on the sample type.

The **COPAS VISION™ Instrument with FlowPilot™ software** adds brightfield imaging to our large particle sorting capabilities. As with our original COPAS platform of flow cytometers, the COPAS VISION provides automated high-throughput analysis and sorting of viable multicellular organisms, cell clusters, bead-like particles and other sample types that are too large or too fragile for traditional flow cytometers.

COPAS VISION has expanded on these capabilities in several ways, most noteworthy is the ability to capture images of the objects in the sample.

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 VISION** large particle cytometer allows you to study the cell-cell interactions found in tissues, tumors or organoids without the need to disrupt the clusters. If you are working with model organisms, replacing manual sorting with a **COPAS VISION** instrument provides fast, sensitive, reproducible automation for gentle sorting and high-throughput screens.

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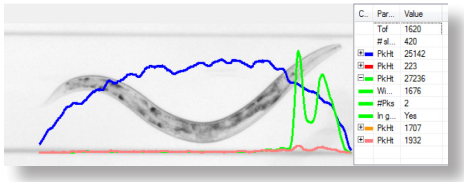
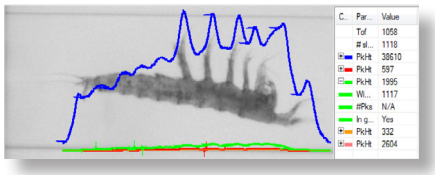
Large Particle Analysis and Sorting

Imaging in Flow

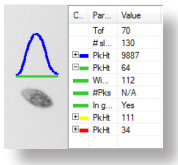
The **COPAS VISION** provides image capture capabilities on a flow cytometry platform. These images reveal the identity of the different particles in the analyzed sample. Many sample types are composed of morphologically similar objects and having representative images is less important. However, other sample types are mixtures of different kinds of particles or organisms, variously shaped, and passing through the flow cell in random orientation. For these the accompanying image informs the flow cytometry data with additional information. For example, meiobenthic samples contain a variety of small invertebrate organisms, many similar in size but biologically very different from each other, and this is easily revealed by the collected brightfield images. Another sample type that benefits image collection is cell clusters developing into organoids and organoid-like tissues that can take on a variety of shapes and sizes that are difficult to distinguish by conventional flow cytometry data alone.



C.elegans adult



Copepod



Paramecium

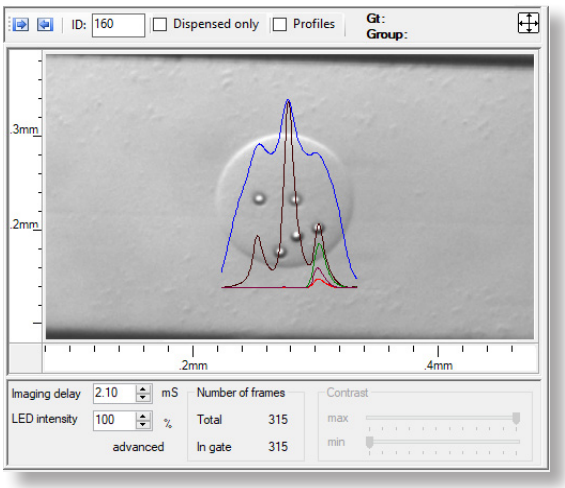
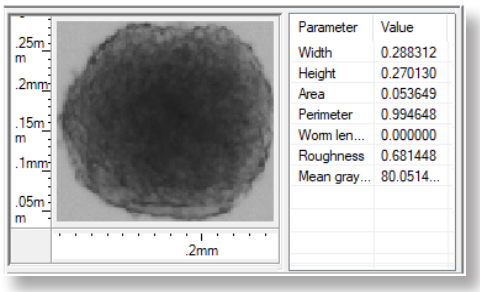


Image capture of encapsulated beads

The flow cytometry data collected by the COPAS VISION consists of histograms and various dot-plots on which regions can be selected for analysis and dispensing. The addition of images from selected regions provide immediate object identification to the researcher. Certain samples composed of particles that are asymmetric or bilaterally symmetric generate data that depends on the orientation of the object as it passes through the flow cell. Analysis of the images provides a method to more accurately determine the number and concentration of each type of particle, measurement of morphological characteristics, confirms the identity of the sample particles, and documentation that can be used for quality control in applications where verifying the identity of a dispensed object is required.

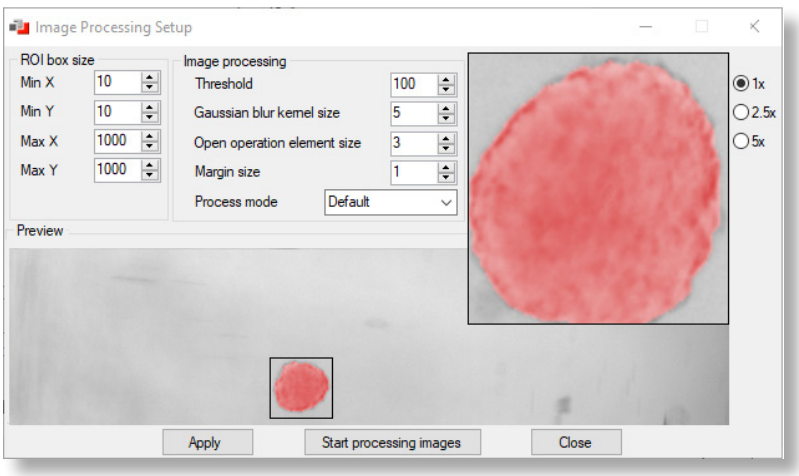
Image Collection and Analysis

Images are collected on the COPAS VISION from a position subsequent to flow cytometry data acquisition. These images can be analyzed post acquisition with FlowPilot image processing software. A region of interest (ROI) is determined for the collected image and measurements of width, height, area, perimeter, axial length, roughness and mean grey scale is determined.



cell clusters

The data can be viewed and displayed in a number of different ways, either as a collection of all ROIs or each image separately with its accompanying metrics. Data derived from image analysis may be stored as well.

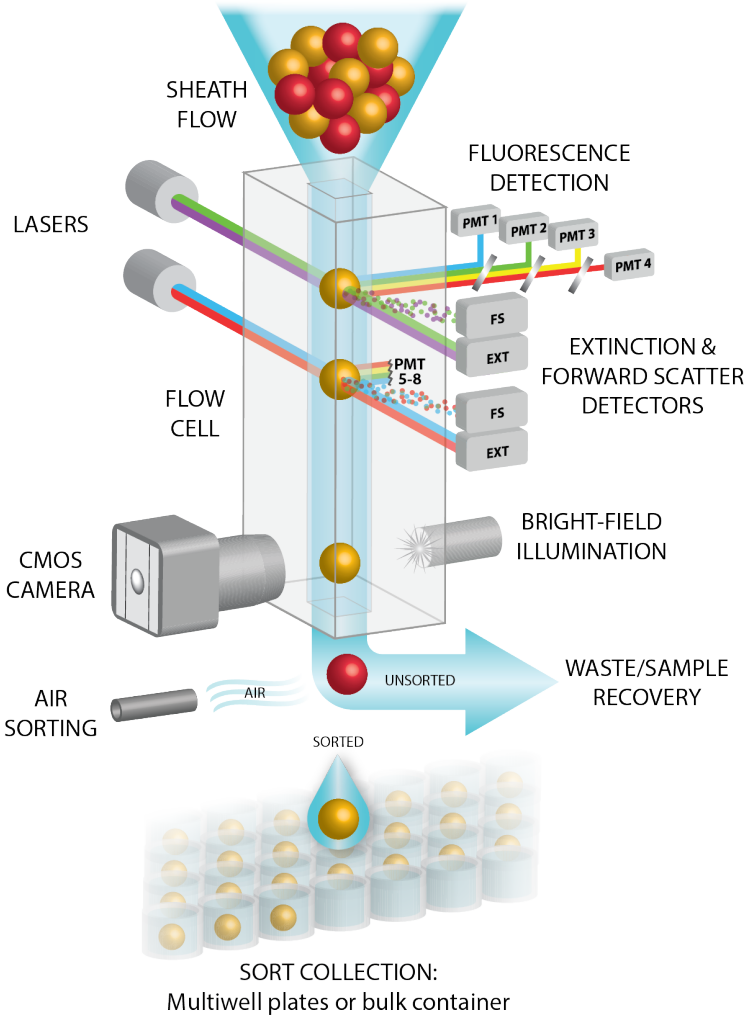


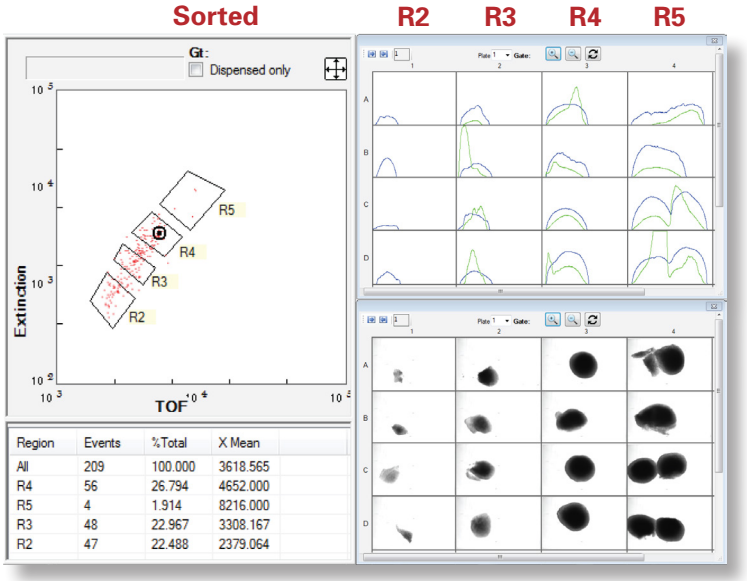
Patented, Gentle Sorting Mechanism

Samples travel from a continuously stirred sample cup to a flow cell where they are enveloped by a sheath solution which hydrodynamically focuses them into the center of the stream for interrogation by up to four lasers. The combined fluid stream exiting the flow channel is continuously diverted by an air stream to a waste/recovery container unless a 'sort' signal is produced. In that case, the air diverter is briefly turned off to generate a droplet of fluid containing the sortable object which then falls directly below the exit nozzle into a collection vessel of the operator's choosing.

The COPAS VISION platform is based on the fundamental principles of flow cytometry but differs from traditional flow cytometers in several important design areas:

- **First,** the large-bore fluidics of the COPAS VISION instruments can accommodate objects as wide as 10–1500 microns, a range that is much larger than traditional flow cytometers.
- **Second,** COPAS VISION systems operate at slower flow rates and lower pressures thereby avoiding the potentially disruptive high shear forces inherent in standard flow cytometers.
- **The third** difference is the heart of our COPAS technology. A 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.





Samples provided by Baranov lab, Schepens Eye Research Institute, MGH, Boston, MA

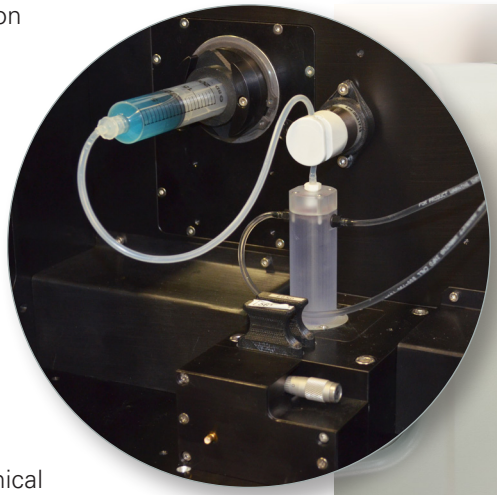
The instrument operator can select to sort all particles in a sample or define a gate region to sort only a subfraction of the entire population, only those that meet certain sort criteria. An example of gated regions and the resulting sort is shown in the panel on the left. Regions R2, R3, R4, and R5 are defined by the operator on the TOF vs EXT dot plot. COPAS VISION dispenses particles matching one criterion at a time. Single cell clusters were dispensed to wells of a multiwell plate, first from the R2 regions, then subsequently from the other three regions. The Profiler data and the image for each cell cluster is displayed. The Profiler data shows the specific pattern and level of fluorescence expression, while the brightfield image shows morphological features for each of the different cell clusters.

Sample Introduction

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

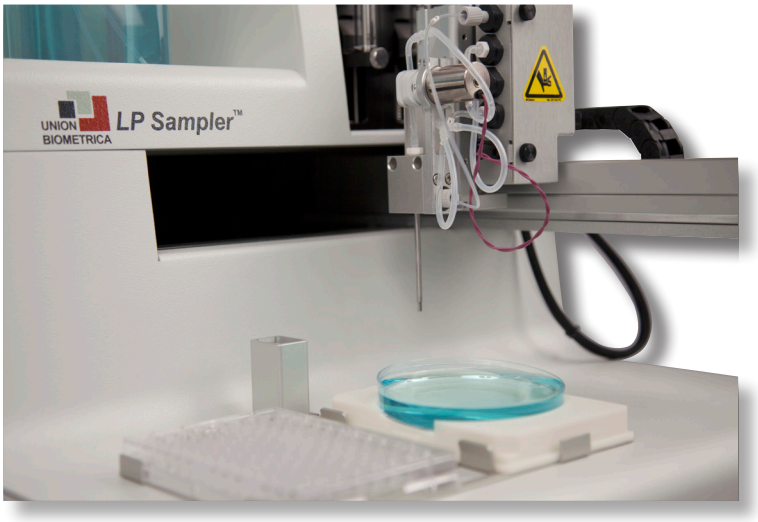
An optional 750 ml stirred sample cup is available for larger volume samples as needed in large screens.

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 within a disposable syringe without mechanical stirring.



OSIS introduction chamber

Stirred sample introduction cups



LP Sampler

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

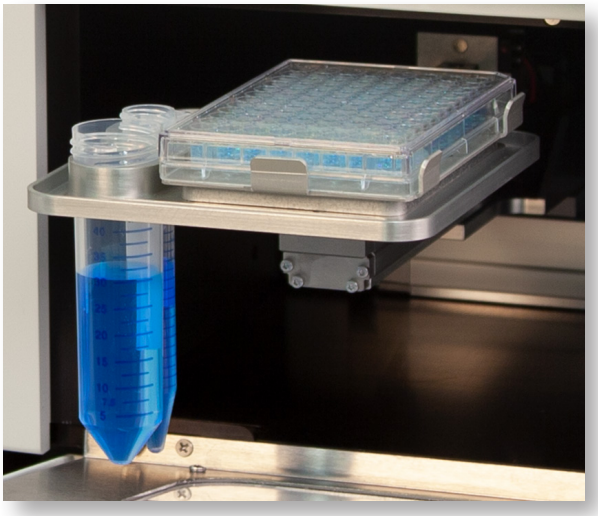
Sample Output

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.**

With COPAS VISION, dispensing is not limited to standard multiwell receptacles. The user can create custom output receptacle templates. Each dispense location may be given a different combination of sort conditions (i.e., object number and gate region).

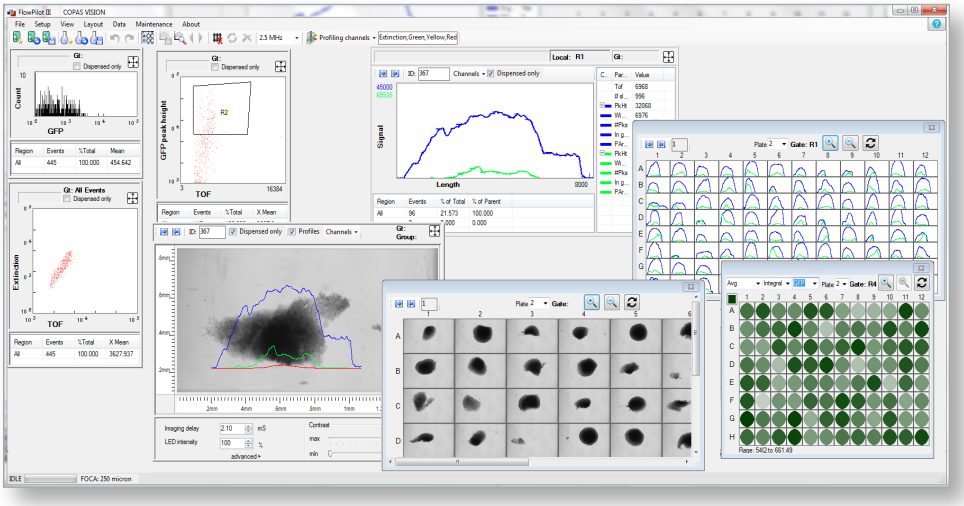
Integration

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



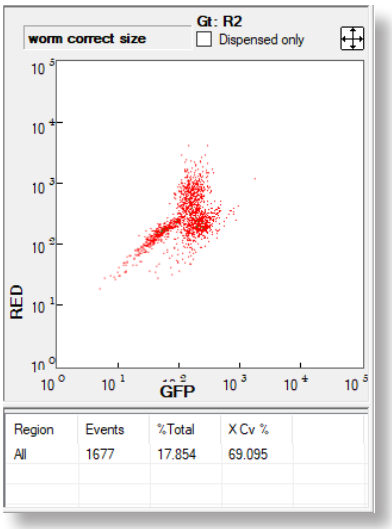
Dispensing stage for sorting

FlowPilot™ Software for System Control & Data Analysis



Union Biometrica's **FlowPilot software** was developed for COPAS VISION and FP 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.

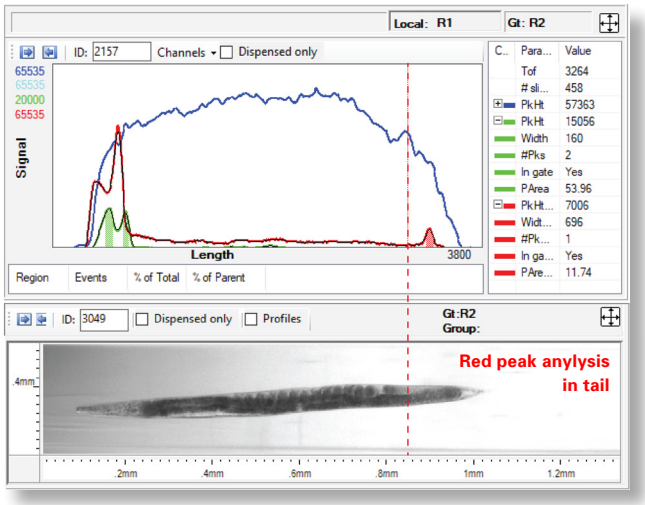


Profiler Feature

Profiler takes data collection to the next level by simultaneously recording the intensities of extinction, forward scatter 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 inside 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 shows 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 ratio-metric analysis—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 and fluorescence measurements exclusively to the organism's head, tail, middle, or ends region.

The example at right shows identification of red fluorescence in neurons in the tail 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).*



Partial Profiling of C.elegans adult

Data Review

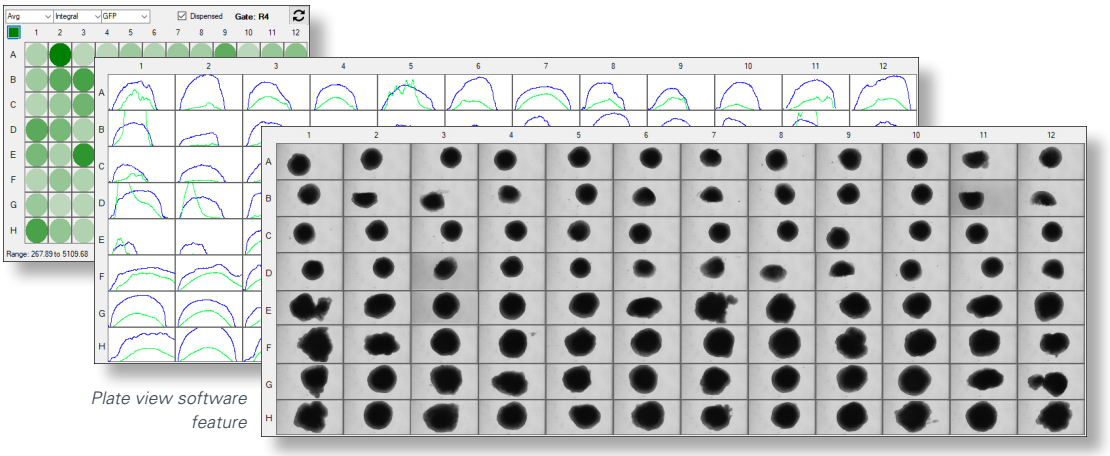


Plate view software feature

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 or 2000 µm

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

Multi-laser Configurations

Up to four (4) lasers can be configured depending on the particular fluorophores to be analyzed.

Single or Dual Detection Modules

1 or 2 focal spots direct light to 1 or 2 detection modules to collect extinction, forward scatter and four channels of fluorescence. Each module contains 2 PIN photo-diodes and 4 PMT detectors. Swappable optical mirrors and filters allow customization of detection across the visible spectrum.

	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

Large Particle (LP) Sampler

Sample Introduction

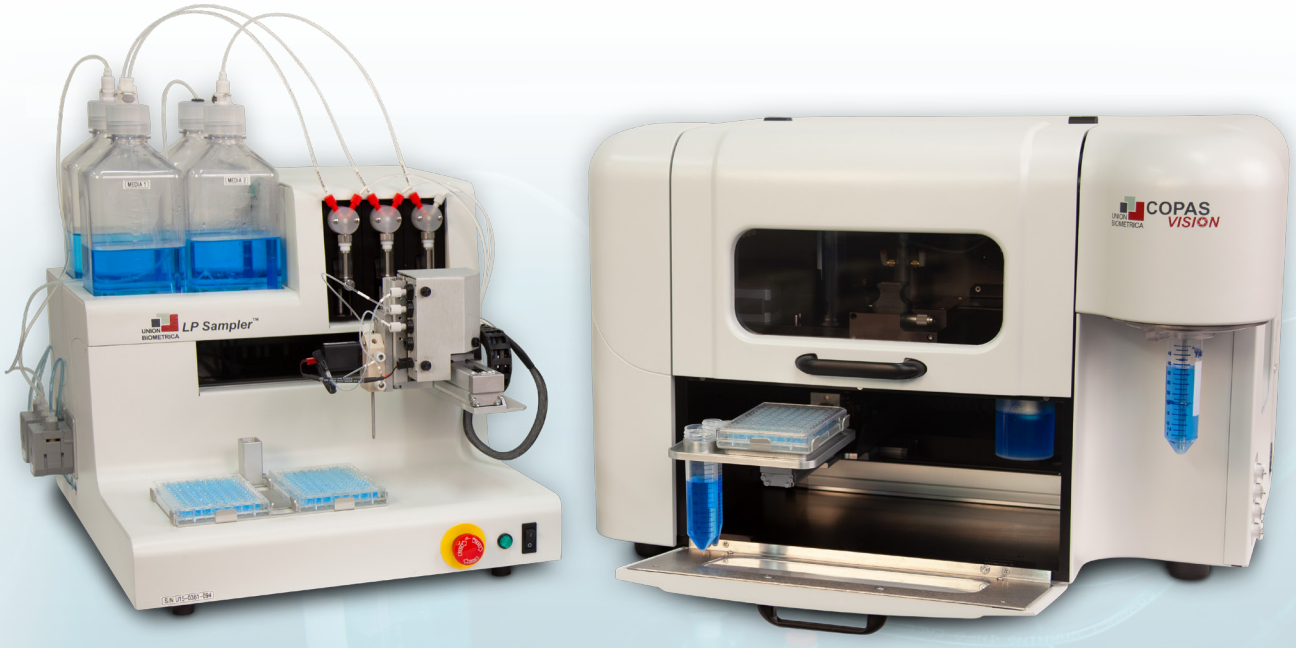
Choose stirred sample cups of 50 or 750 ml sizes. The OSIS chamber can be used for extremely delicate samples or samples that float or sink.

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 VISION system.

Integration Feature

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



Large Particle (LP) Sampler with COPAS VISION

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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