BioSorter®

Multi-Range Large Particle Flow Cytometer for 10-1500 Micron Objects

An expandable platform with interchangeable flow cells for automated high throughput analysis and sorting of viable multicellular organisms, cell clusters, bead-based libraries and other samples that are too large or too fragile for traditional flow cytometers.

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. But researchers studying larger objects were historically limited to manual manipulation under a microscope – a technique that is tedious, error prone and severely limits throughput. So there was a need for high throughput sorting technology 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 of 250, 500, 1000 or 2000 micron diameter.

The **BioSorter® Instrument with FlowPilot™ software** provides the next level of capabilities **for individual laboratories where ultimate system performance, maximum flexibility and ease of use are important**, such as with high throughput screening.
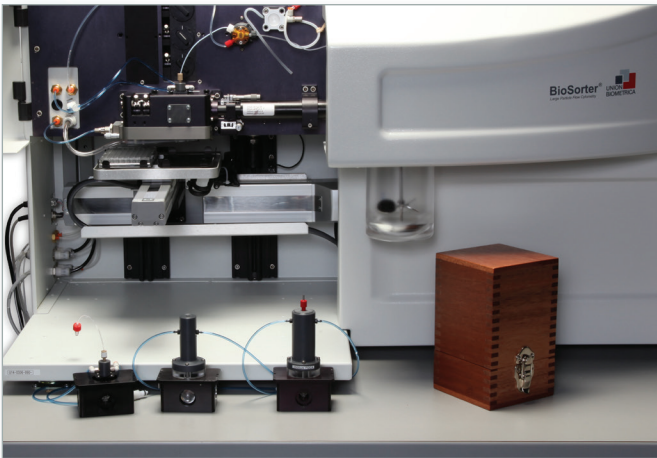
With its interchangeable FOCA (Fluidics and Optics Core Assemblies) the BioSorter combines the capabilities of all four COPAS models into a single platform which can accommodate samples across an expanded range of 10-1500 micron sample diameters. This flexibility is also well suited for instruments to be **shared between multiple labs or in core service labs**.

If you are studying multicellular structures you know that once cells form into clusters they communicate and behave differently than in isolation. The BioSorter large particle cytometer allows you to **study the cell-cell interactions found in tissues, tumors or organ systems without the need to disrupt the clusters** for traditional analysis. Or, if you are working with model organisms replacing manual sorting with a BioSorter instrument provides fast, sensitive, reproducible automation for gentle sorting and high throughput screens.

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Large Particle Analysis and Sorting
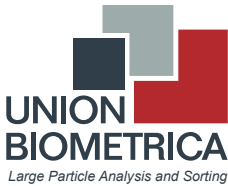
Unique Interchangeable FOCA

The BioSorter instrument is a continuous flow system capable of analyzing, sorting and dispensing objects ranging in size from 10 to 1,500 µm. This broad range is made possible by the BioSorter's unique **Fluidics and Optics Core Assemblies (FOCA)**.
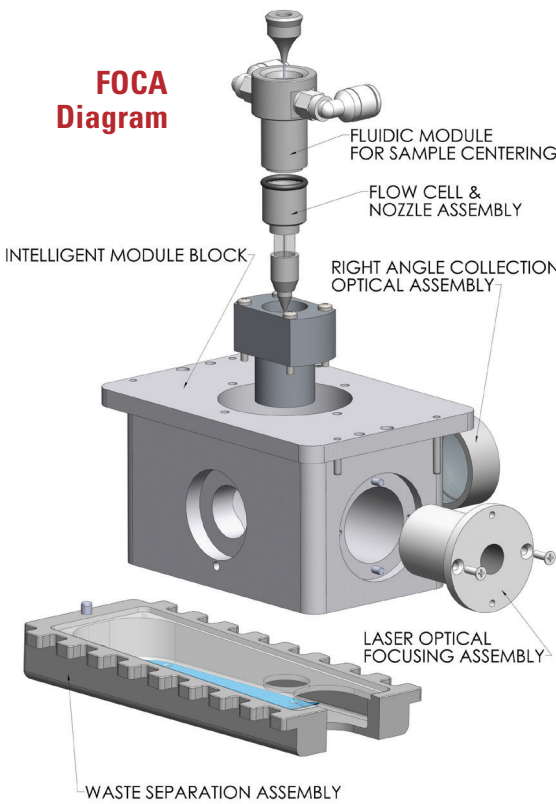

FOCA with pre-aligned optics make changeover easy

FOCA Diagram

- FLUIDIC MODULE FOR SAMPLE CENTERING
- FLOW CELL & NOZZLE ASSEMBLY
- INTELLIGENT MODULE BLOCK
- RIGHT ANGLE COLLECTION OPTICAL ASSEMBLY
- LASER OPTICAL FOCUSING ASSEMBLY
- WASTE SEPARATION ASSEMBLY

Because each FOCA is a precision assembly of fluidics, flow cell and its corresponding pre-aligned optics, an operator can quickly and easily change between samples of different size ranges.

A few examples of FOCA choices for various samples are shown at right. Our application scientists can help you refine your choice of FOCAs based on their extensive experience. Additional FOCA units can always be added later. *(Ask about custom units)*

	FOCA-250	FOCA-500	FOCA-500 metal-free	FOCA-1000	FOCA-2000
Recommended Object Size	20-150 µm	40-300 µm	40-300 µm	200-700 µm	500-1500 µm
Typical Examples	• C. elegans • iPSCs	• Drosophila embryos • Plant protoplasts	• Mammalian adipocytes	• Islets • Arabidopsis	• Zebrafish embryos & larvae

Up to 4 excitation lasers

BioSorter systems can be configured with up to four lasers. Relative axial length (TOF, Time of Flight) is measured by an axial light-loss detector. Optical density (EXT, Extinction) is determined by the total integrated signal of the light blocked. The fluorescence emission can be simultaneously measured at three different PMT detectors at wavelengths determined by interchangeable emission filters and dichroic mirrors.
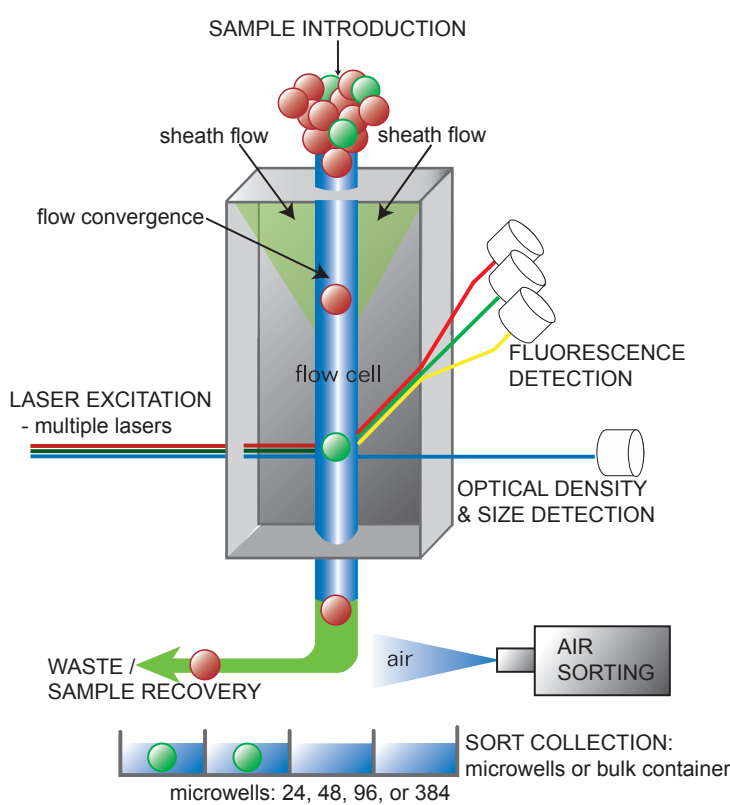
Patented, Gentle Sorting Mechanism

Samples travel from a continuously stirred sample cup to a flow cell where they are surrounded by a sheath solution which hydrodynamically focuses them into the center of the stream for interrogation by up to four lasers. By default, all fluid exiting the flow channel is 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.

The BioSorter 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 and BioSorter FOCAs can accommodate objects as wide as 10 – 1500 microns, which is much larger than the traditional flow cytometers.

- **Second**, BioSorter 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 the COPAS and BioSorter 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.

Taken together, these design features of the BioSorter system permit high speed analysis and gentle sorting of large objects. This gentle handling maintains viability while delivering high recoveries of purified biological materials.

SAMPLE INTRODUCTION
sheath flow   sheath flow
flow convergence
flow cell
LASER EXCITATION - multiple lasers
FLUORESCENCE DETECTION
OPTICAL DENSITY & SIZE DETECTION
WASTE / SAMPLE RECOVERY   air   AIR SORTING
SORT COLLECTION: microwells or bulk container
microwells: 24, 48, 96, or 384

Some 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	• Bead Based Assays	• *C. elegans*	• *Arabidopsis & Nicotiana* seeds
• Cardiomyocytes	• Cells in & on beads	• *D. melanogaster*	• Calli
• Duct Cells (Kidney, Pancreatic, etc.)	• Encapsulated samples	• Marine Plankton	• Fungi
• Pancreatic Islets	• Microspheres	• *Medaka*	• Pollen
• Stem Cell Clusters / EBs		• Mosquito	• Protoplasts
• Spheroids & Organoids (mammary, neurospheres, intestinal, tumorspheres)		• Zebrafish (*D. rerio*)	

Sample Introduction

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

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

A flow-through 5 ml rotating sample introduction chamber is available. It is designed specifically for handling extremely delicate samples (e.g. adipocytes, plant protoplasts) and for samples that tend to either float or sink in a sample cup with normal stirring-type agitation.
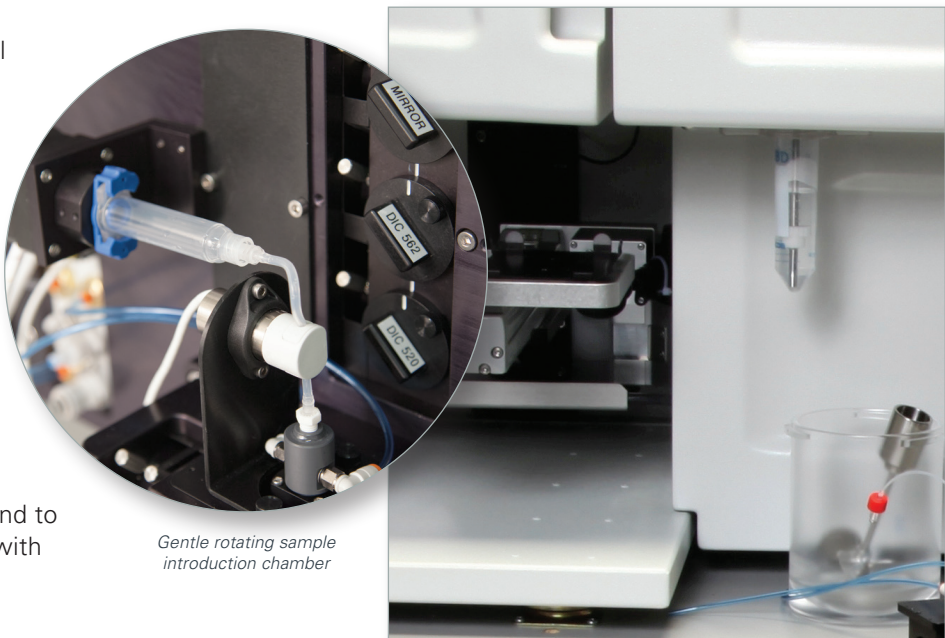
Gentle rotating sample introduction chamber

Choices of stirred sample cups
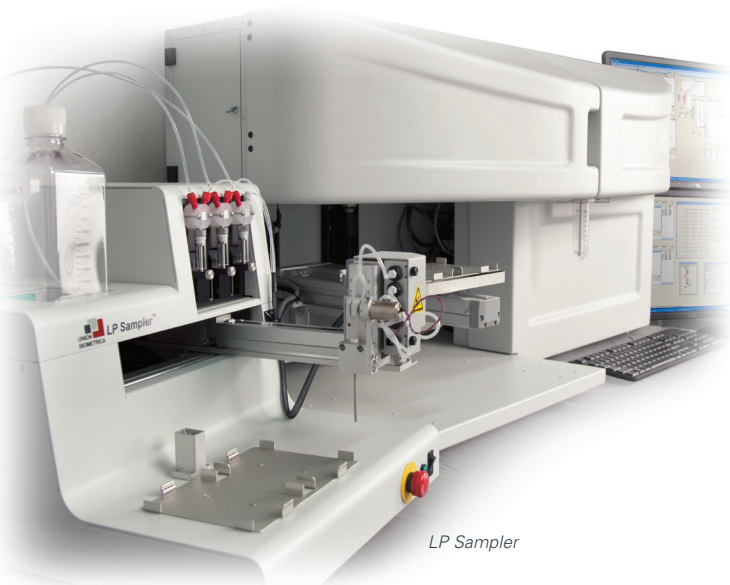
The **Large Particle (LP) Sampler™** can introduce the full range of 10–1500 micron samples from wells of multiwell plates, petri dishes, microfuge tubes and other similar sample containers.

LP Sampler

Sample Output

The X-Y-Z stage allows dispensing into 24-, 48-, 96-, or 384-well multiwell plates, tubes and bulk receptacles. The Z-direction allows adjustment for different height plates and tubes.

With BioSorter 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).

*Flexible X-Y-Z stage for dispensing*

Integration

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

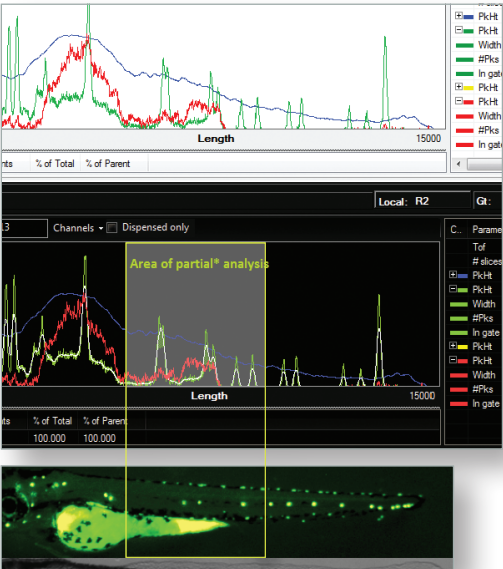# FlowPilot™ Software for System Control & Data Analysis

Union Biometrica's **FlowPilot software** was developed for BioSorter® and COPAS FP instruments with the demanding flow cytometry user in mind. But the software is intuitive and easy-to-use so you don't have to be an expert to begin using FlowPilot equipped instruments.

The dynamic Flow Pilot 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 tight 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.
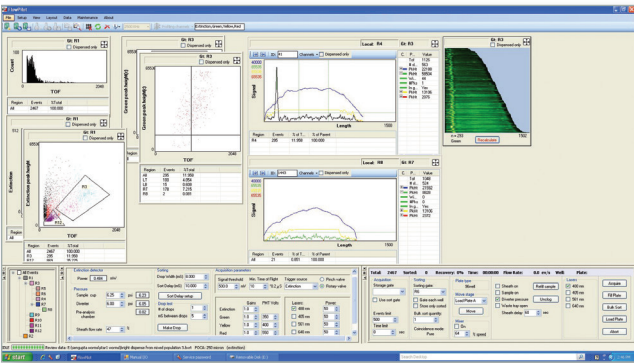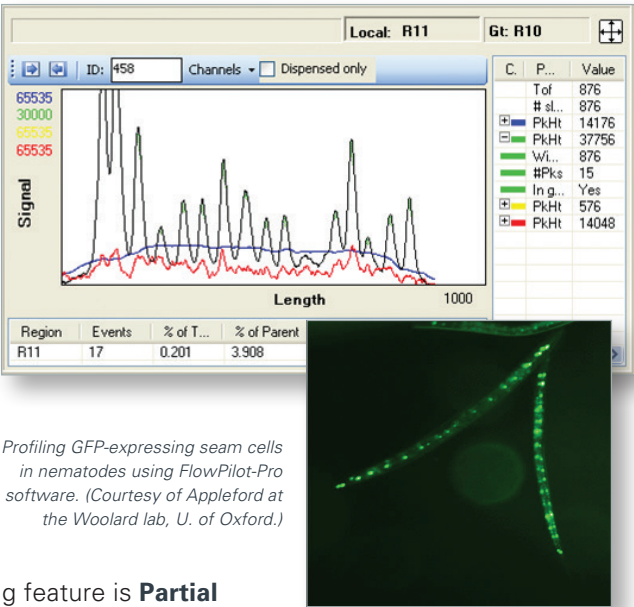
Profiler II/FlowPilot-PRO™ Software

Profiler II, unique to Union Biometrica, takes data collection to the next level by simultaneously recording up to a maximum of 8,000 measurements along each object's time of flight (TOF) for each of the four optical parameters: extinction and three fluorescence channels. The software then graphically and numerically displays variations of those signals along the length of an object as a succession of peaks and valleys that directly trace the fluorescence intensity and optical density of the object as it passes through the laser in the flow cell. The result is an "optical profile" of each object graphically mapping the location and intensity of all four optical parameters plus determining values for peak height, peak width, peak count and relative position – all of which can be used as sort criteria.

Profiling GFP-expressing seam cells in nematodes using FlowPilot-Pro software. (Courtesy of Appleford at the Woolard lab, U. of Oxford.)

Partial Profiling of a zebrafish larva.

Another profiling feature is **Partial Profiling**. By zeroing in on one region of the profile, Partial Profiling allows you 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 (**pp**). Partial Profiling can be configured to analyze extinction and fluorescence measurements exclusive to the organism's head, tail, middle, or end regions.

For example, presence of green**pp**[*head*] HEAD NEURONS vs red**pp**[*center*] OVARY regardless of any other green or red fluorescence in the animal). (***pp** indicates partial profiling is active in the head area [*head*] or center of object [*center*]).

Summary Of Available Options

FOCAs to Cover the Entire 10-1500 Size Range
Choose one or more sizes to match your experiments.

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

FlowPilot PRO Profiling Software Feature
This software/hardware combination digitizes the object into a succession of peaks and valleys that directly traces the optical density and fluorescence pattern along the axial length of the object. Profiler can collect up to 21 parameters per object and use these measurements as sorting criteria.

Sample Introduction
Choose stirred sample cups of 40, 750 or 1500 ml sizes. A rotating 5 ml 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 BioSorter system.

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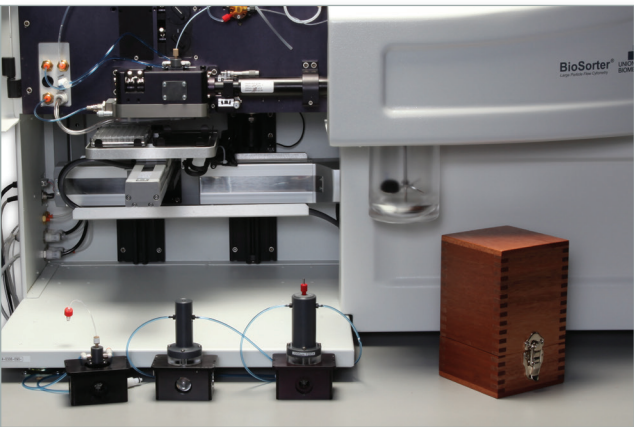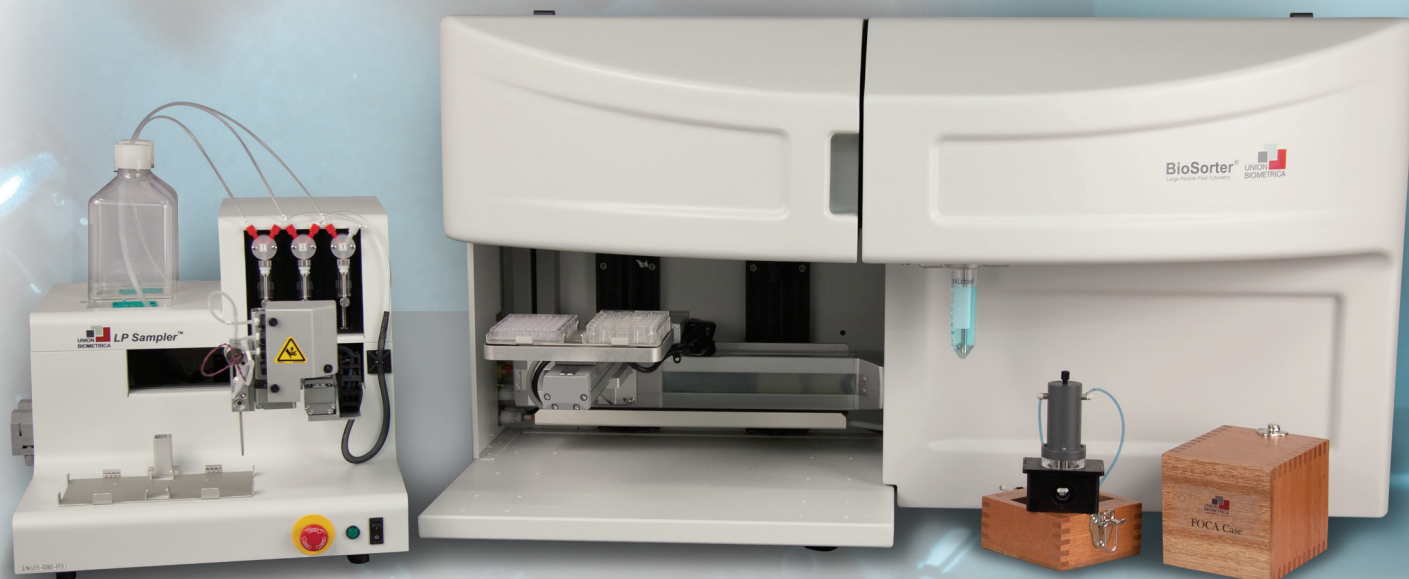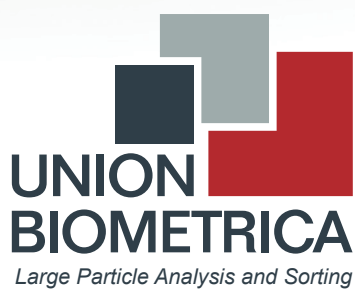

FOCAs in various size ranges
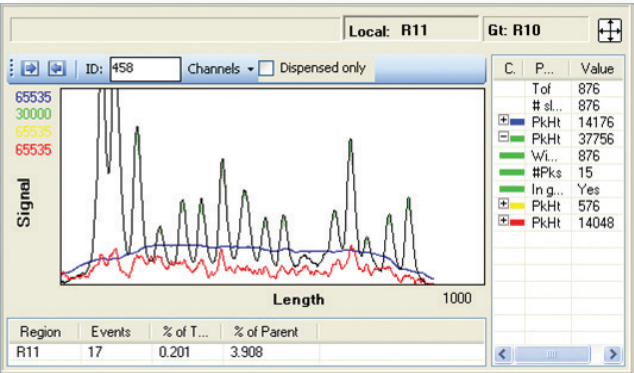
Profiler II/FlowPilot-PRO™ Software

Large Particle (LP) Sampler

Large Particle (LP) Sampler with BioSorter and FOCA

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