UNION BIOMETRICA
Large Particle Flow Cytometry

Profiling Software

Upgrading from the standard FlowPilot™ software to FlowPilot-Pro™

Expand the Capability of your COPAS™ and BioSorter® Systems by Mapping Fluorescence Positional Information

Which would you rather have for your research?

Think about the fundamental differences between analyzing simple single cells vs. the analysis and sorting of relatively large cell clusters like embryoid bodies, multicellular model organisms such as nematodes or zebrafish, or gel micro-capsules containing monoclonal bacterial colonies.

For typical single cell cytometry or even homogeneous large objects, obtaining a single data point for each parameter / channel is sufficient. But most large objects are NOT homogeneous in terms of fluorescence, optical density, etc.

Would you like to have a map of fluorescence intensity across the entire object? We call those maps “Profiles”. Would you want to be able to sort based on those Profiles? Would it be helpful to have user definable sort criteria for profile peak heights, widths, integrals, locations and number for each optical parameter – providing up to 21 user selectable values? These features are unique to large object flow cytometry by Union Biometrica.

We invite you to explore the world of Profiles and **FlowPilot-Pro**

FlowPilot-Pro

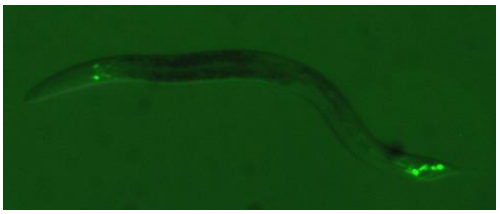
The **FlowPilot-Pro** option package can take your sorting and analysis decisions to an entirely new level.

What if you could . . .

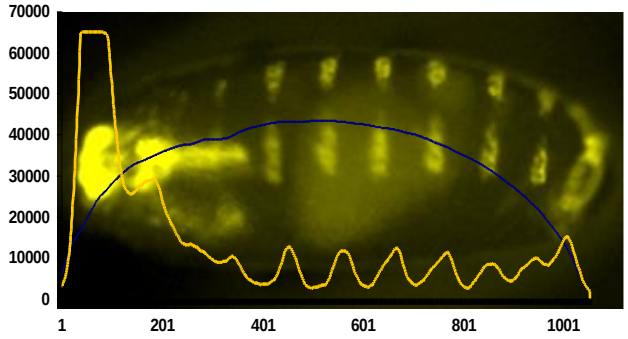
- Record up to 8,000 optical slices per model organism (or other object) thereby elucidating cell or organ level positional information?
- Detect weak fluorescence signals even in the presence of much stronger signals?
- . . . or in the presence of background auto-fluorescence?

Like most flow cytometer software, the standard FlowPilot software provides a single integrated signal measurement for each parameter of an object, and these signals are used for analysis and for sorting decisions.

The **FlowPilot-Pro** option package takes this to the next level by simultaneously detecting and recording up to 8,000 data points per object for each of the four optical parameters: extinction and three fluorescence channels.



Nematode with green fluorescent marker expressing in both head and male tail. (Strain provided by Maureen Barr.)



Banded Drosophila Embryo (head on left). (Transgenic Drosophila strain provided by Greg Beitel.)

FlowPilot-Pro digitizes 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. **FlowPilot-Pro** then graphically and numerically displays those variations in fluorescence and extinction intensity along the length of an object. The result is an optical profile of each object graphically showing the location and intensity of all four optical parameters. Profiling also enables users to optimize COPAS & BioSorter systems by visualizing data, resulting in better detection of strong versus weak signals.

FlowPilot-Pro offers:

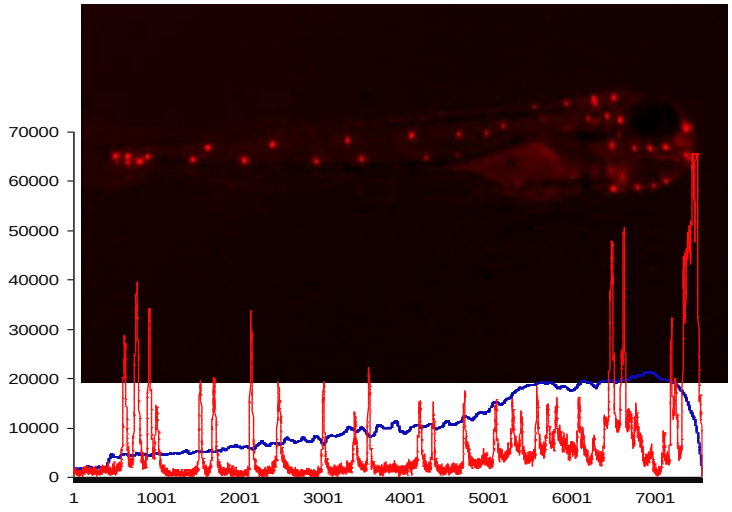
- 4 channels of simultaneous profiles, one for each of the 4 optical parameters (Extinction + 3 channels of fluorescence)
- Independent channel scaling
- Enhanced sorting based on user-definable sort criteria for:
  - Peak height
  - Peak width
  - Relative peak location
  - Number of peaks for each parameter

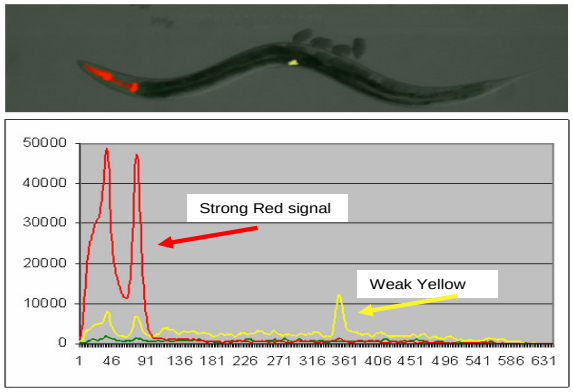
Examples of how some researchers are using Profiler

Mapping the Fluorescence Profile of a Zebrafish

Axial profile (red-fluorescence and blue-extinction, shown at right, of a stained 4-day old wild type zebrafish larva overlaid on the corresponding image.

This is an example where sorting could be based on **Number of Peaks for each Parameter.**





Detection of weak fluorescent signals even in the presence of much stronger signals

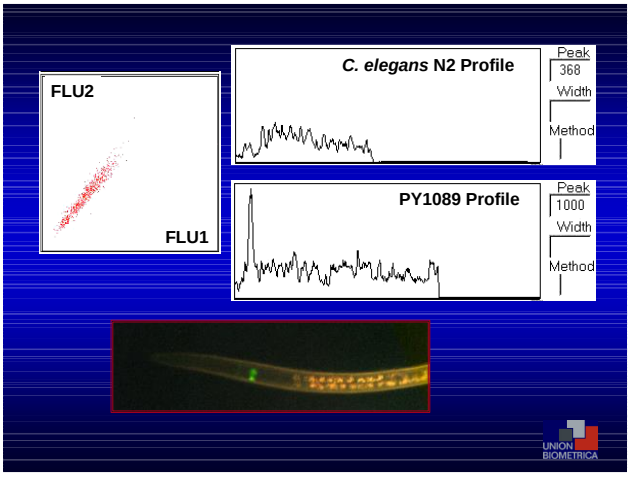
The figure shown at left demonstrates that without the positional data, the presence or absence of the weaker signal would just be lost or buried in the "average" presence of the stronger signal.

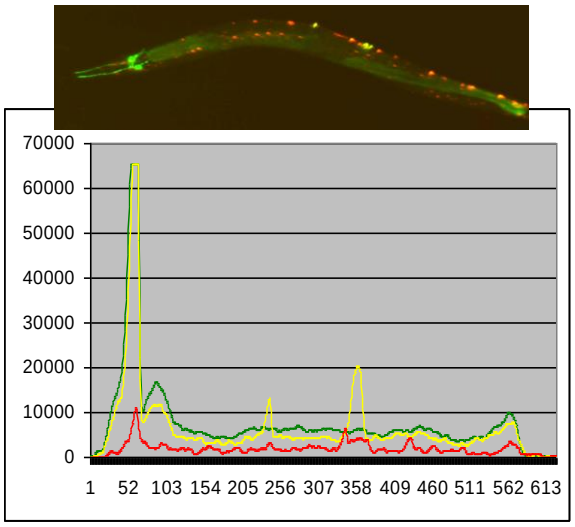
This is an example of sorting based on **Relative Peak Location**.

Detection of Weak Localized Fluorescence vs. Auto-fluorescence (Background)

In the figures shown at right, when using the basic COPAS or BioSorter for this type of experiment, notice that a mixture of two population types (N2 and PY1089) are not differentiated on the scatter plot graphing FLU1 (green fluorescence) and FLU2 (red fluorescence or auto fluorescence). Profiling, however, provides enhanced sensitivity and enables detection of a weak localized fluorescence signal even against an auto-fluorescence background, showing the two population types on the line graphs on the right.

This is an example where sorting can be done on the basis of **Peak Height above a Threshold.**

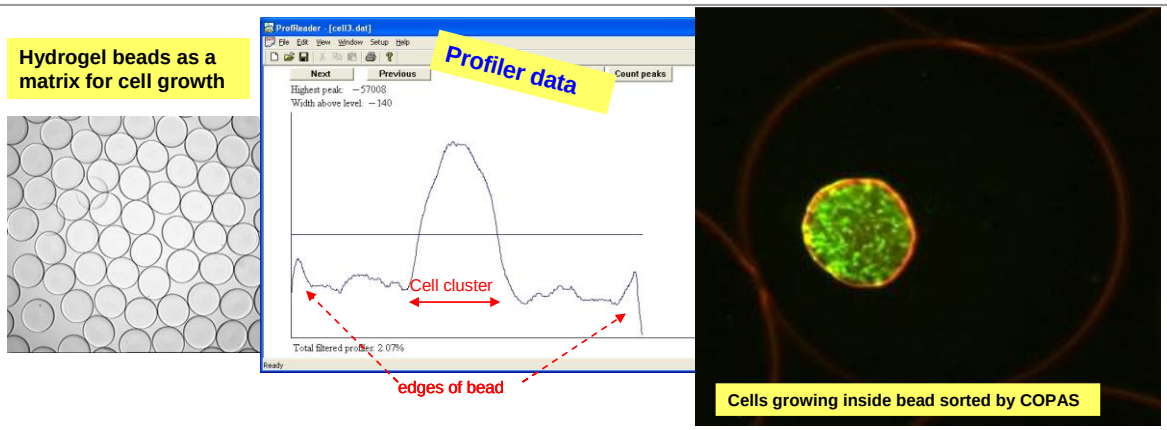




Three-Color Profiling

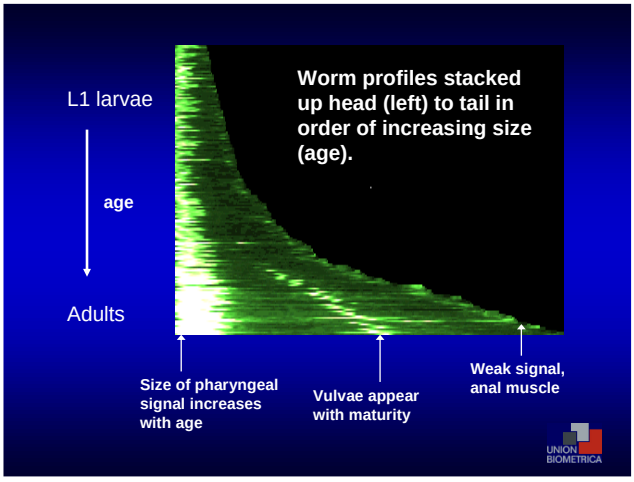
The KS329 strain (shown at left) was constructed and kindly provided by Yanping Zhang and Mike Herman of Kansas State U. The GFP expression in the head neurons and the yellow protein expression in the vulva can be used as landmarks for assessing the position of the cells expressing red fluorescing protein from the mab-5 promoter.

This is also another example of sorting based on **Relative Peak Location**.



Cell clusters growing inside Hydrogel beads

Beads formed around cells can be inspected by **FlowPilot-Pro**. COPAS can then be used to sort beads containing only one cell. As these cells grow & divide they form monoclonal clusters. (Courtesy of S.Panke and M.Walser, ETH, Zurich.) Here sorting can be accomplished based on **Peak Width** as a sign of cell proliferation.



Profiling Expression Throughout Development

For a single gene, the change in expression through development can be shown in terms of the amount of expression, when expression begins, or dim expression. Figure at left shows examples of these expression terms: amount of pharyngeal expression displayed increases, expression of the vulvae begins to appear on the graph at a certain maturity, and weak fluorescence of the anal muscle is also detectable using this analysis method. A more extensive analysis is described in: DuPuy, *et al.,* Nature Biotechnology, 25, 663-338, 7 May 2007.