Advanced Acquisition and Profiler II™ Options --

Expand the Capability of Your COPAS™ System by Increasing Speed, Sensitivity and Resolving Positional Fluorescence Information

Union Biometrica now offers two optional packages to significantly expand data acquisition and analysis capabilities with your COPAS Large Particle Flow Cytometer.

Advanced Acquisition Package

The **Advanced Acquisition Package (AAP)** includes advanced electronics and enhanced software analytical tools that permit significantly greater data acquisition rates, increased data resolution, and object flow rates.

- Higher signal resolution is achieved with 16-bit data resolution (1:65536) to offer 32 times more detail in intensity and size than the standard COPAS software. Increased resolution makes it possible to distinguish size differences as small as 5 µm.
- Increased sensitivity -- **AAP** also offers the ability to detect smaller objects and sense lower intensity fluorescent signals.
- Higher throughput -- The increased object acquisition rate permits samples to be run four times faster than standard rates.
- Additional software features: 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 3.0 standard.

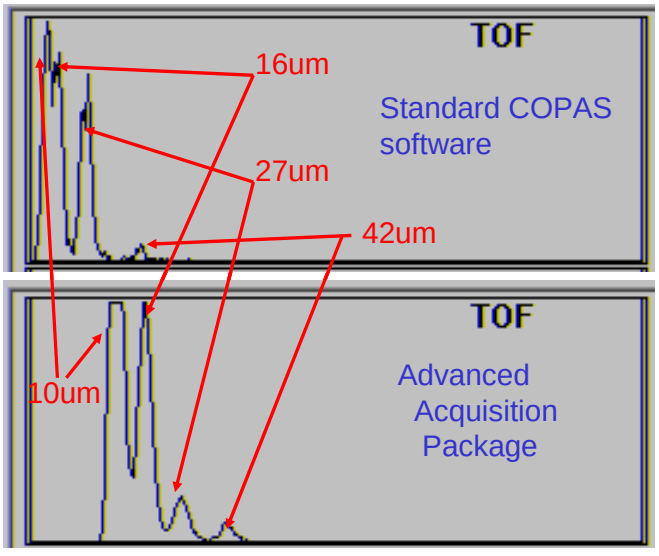

Figure above illustrates improved resolution between 10µ and 16µ particles by using AAP.

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Profiler II

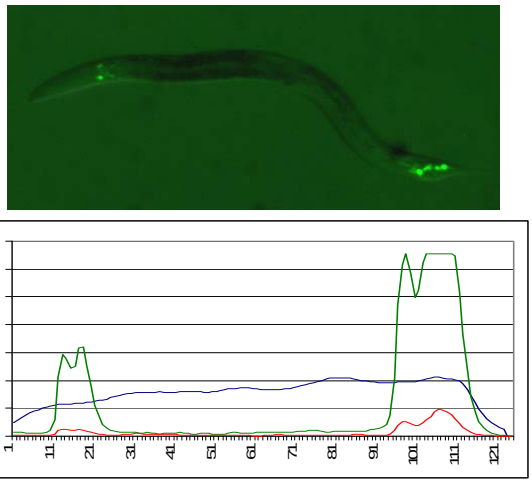
The **Profiler II** option package, which includes and builds upon **AAP**, can take your sorting and analysis decisions to an entirely new level.

What if you could . . .

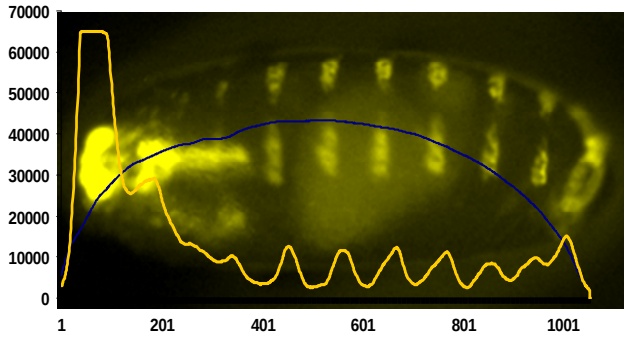
- Record up to 8,000 optical slices per model organism (or other object) thereby elucidating cell or organ level positional information?
- Detect weak fluorescence signals even in the presence of much stronger signals?
- . . . or in the presence of background auto-fluorescence?

Both the standard COPAS software and the more advanced **AAP** provide a single integrated signal measurement for each parameter of an object, and these signals are used for analysis and for sorting decisions.

The **Profiler II** option package (which includes AAP) 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.



Banded Drosophila Embryo (head on left)

Profiler II 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. **Profiler II** also includes advanced imaging to graphically and numerically display subtle 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. Profiler II also enables users to optimize COPAS™ systems by visualizing data, resulting in better detection of strong versus weak signals.

Profiler II 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

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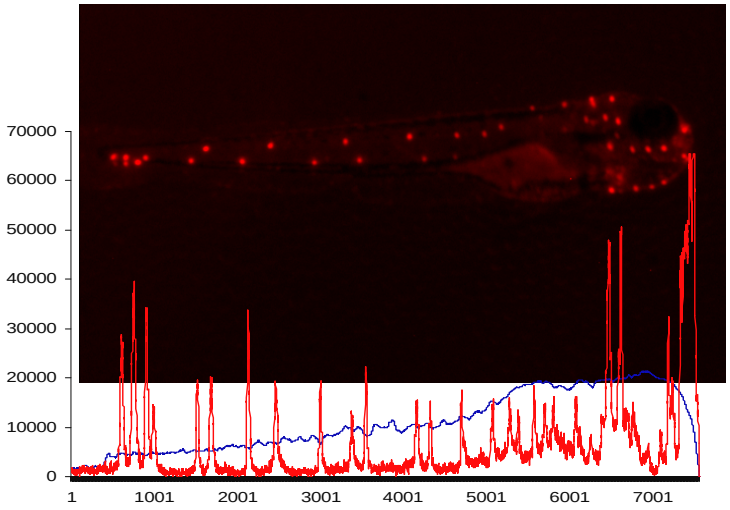
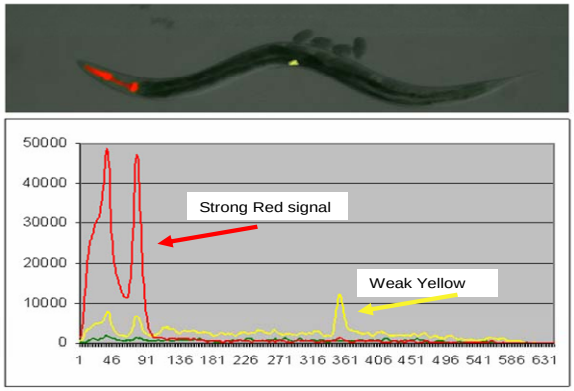
Examples of how some researchers are using Profiler

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

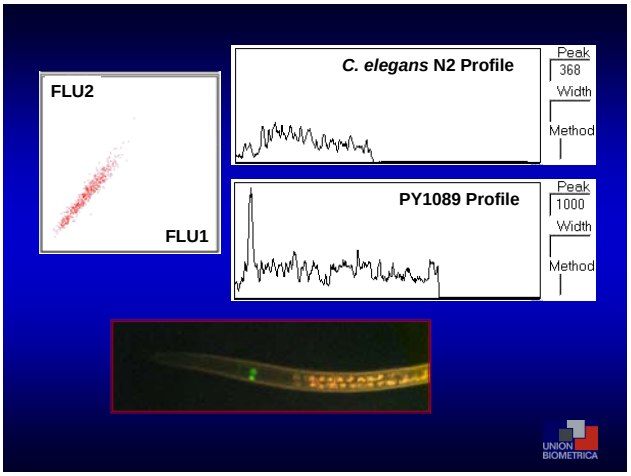
The figure shown at left demonstrates that without the positional data, the presence or absence of the weaker signal would just be lost or buried in the "average" presence of the stronger signal.

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 for this type of experiment, notice that the two population types are not differentiated on the scatter plot graphing FLU1 (green fluorescence) and FLU2 (red fluorescence or auto fluorescence). The **Profiler II** module 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.**



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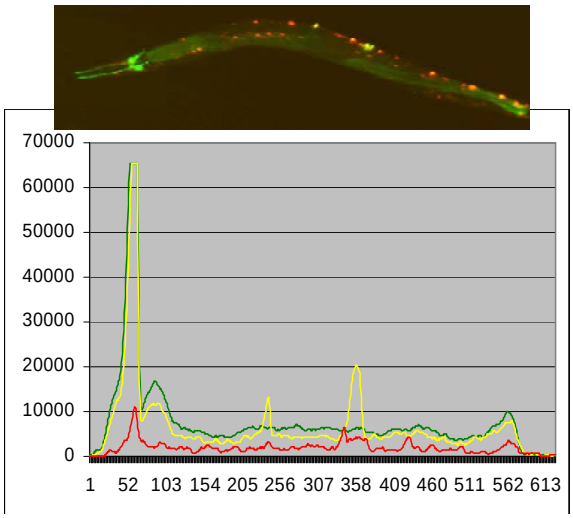
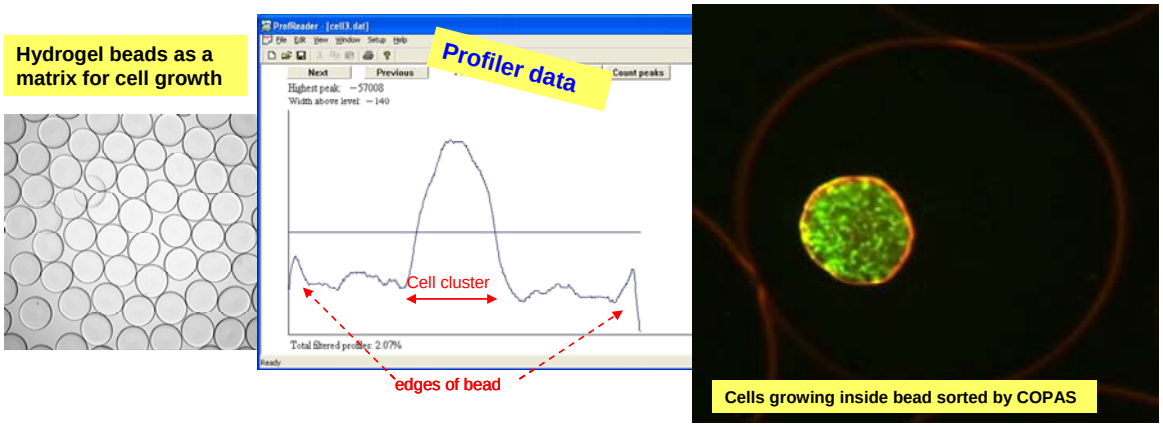


Three-Color Profiling

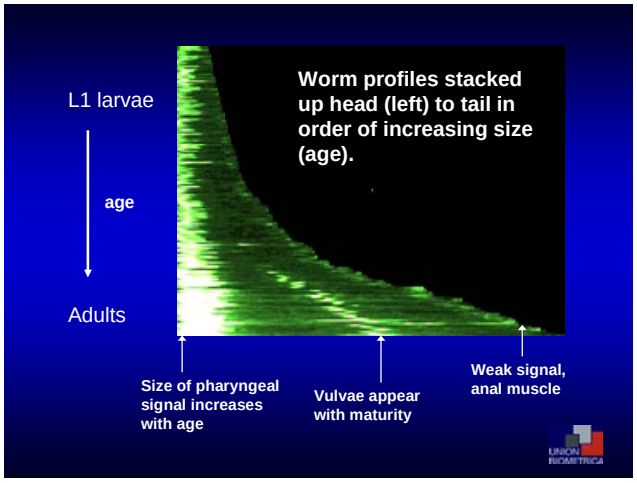
The KS329 strain (shown at left) was constructed and kindly provided by Yanping Zhang and Mike Herman of Kansas State U. The GFP expression in the head neurons and the yellow protein expression in the vulva can be used as landmarks for assessing the position of the cells expressing red fluorescing protein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



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.

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