Adv. Acquisition Pkg. & Profiler II

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Introduction to Advanced Acquisition Package Option

- Advanced Acquisition Package provides users enhanced analytical tools and increased COPAS instrument sensitivity. The AAP includes:
  - Both software and hardware components. Can be added to most existing COPAS systems in the field.
  - Increased data resolution, object flow, and acquisition rates
  - 3 times the current scan rate
  - Improved data displays with linear and logarithmic scaling
  - Improved fluorescence detection with the ability to apply fluorescent compensation
  - Advanced real-time data manipulation to allow user-defined mathematical functions to be applied to object data
  - Extended sorting / dispensing capabilities with user definable sorting regions using logarithmic scales, fluorescent compensation, object coincidence controls, and real-time mathematical functions
  - Expanded data storage capabilities fully compatible with FCS 2.0 standard
  - See following slides for detailed specifications

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Advanced Acquisition Package (AAP) Detailed Specifications

- Increased Object Scan Rate: up to 5MHz (0.2 microsecond)
  - Therefore, a sample stream moving at ~4m/s, COPAS with AAP can sample an object every 0.8um
  - Provides 3 times more detail in TOF (the size measurement “Time of Flight”) resolution
  - Able to detect objects as small as 10um beads on a Biosort
  - Able to detect 1um differences in TOF
  - *Object scan rate on a standard COPAS system (without AAP) is slower at 1.6MHz (0.6 microsecond)*

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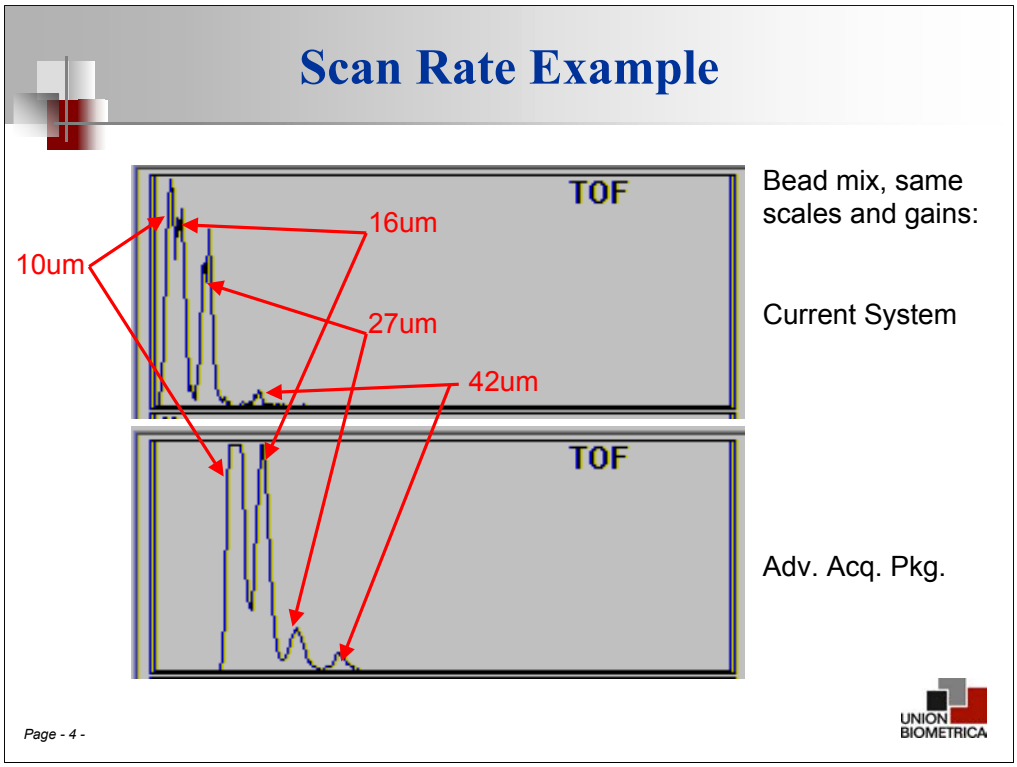
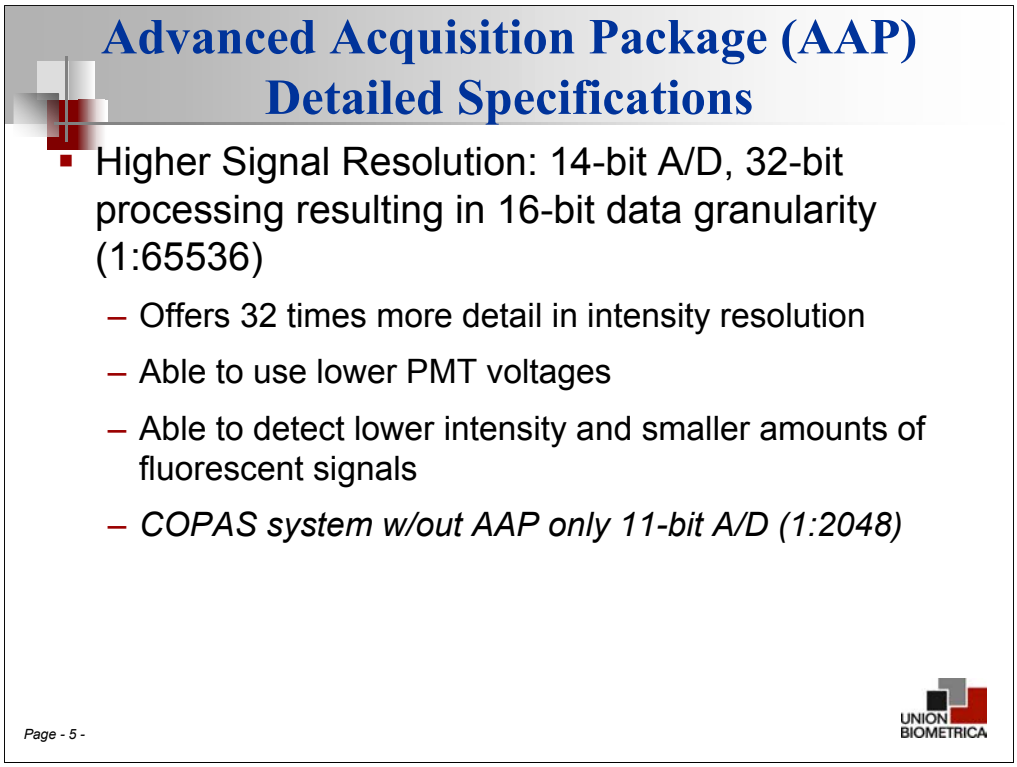
The *Sampling Rate* determines the number of thin sections into which an object can be divided. *Sample Rate* corresponds to the granularity that an object can be examined. Our increased sampling rate allows the COPAS to pinpoint very localized changes in an object’s fluorescence and extinction.



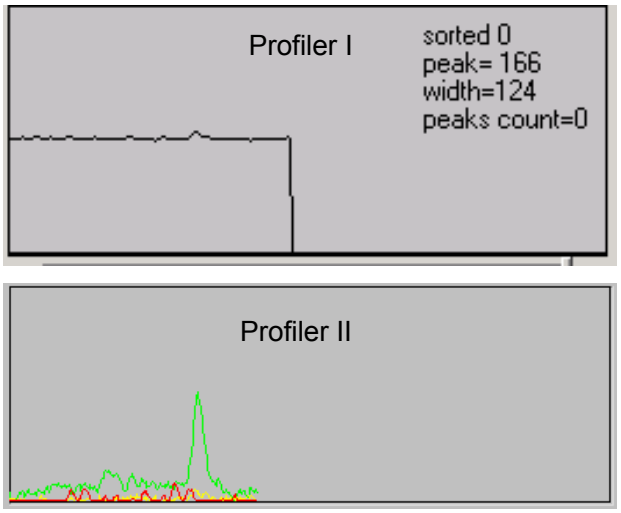
In a standard COPAS System at the standard object scan rate, the system is unable to distinguish subtle differences in object size ~ as shown in the upper graph, the 16 um and 10 um beads are not clearly distinguishable from each other as distinct peaks. Using the COPAS with AAP, the increased scan rate and higher TOF resolution, enables us to distinguish between the two sizes of beads, which can then be sorted.



The *Signal Resolution* determines the range of numerical values assigned to each section. *Signal Resolution* corresponds to the total number of numeric values available for representing optical intensity. Our new 16-bit signal resolution allows the COPAS to detect more subtle variations in an object's fluorescence and extinction.

Signal Resolution Example

C.elegans, *str-1* strain, L2 stage w/ single pair of neurons GFP labeled



Same scale and PMT settings

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Data shown using Profiler I and Profiler II modules. With the higher resolution signal, much more detail is visible using the Profiler II than using Profiler I, making sorting on this low level of fluorescence much more accurate. If run on a standard COPAS without Profiler II, this worm would get lost in the noise of the system due its the low fluorescence. In a dot plot of this worm, using AAP only (no Profiler II), it would still be sortable from a non-fluorescent strain due to the increased system signal resolution.

Advanced Acquisition Package (AAP) Detailed Specifications

- Logarithmic Scaling
  - Able to display small and large objects simultaneously
  - Better discrimination between small objects
  - Tighter populations of large objects

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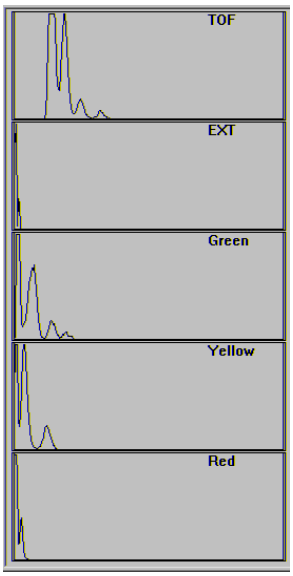
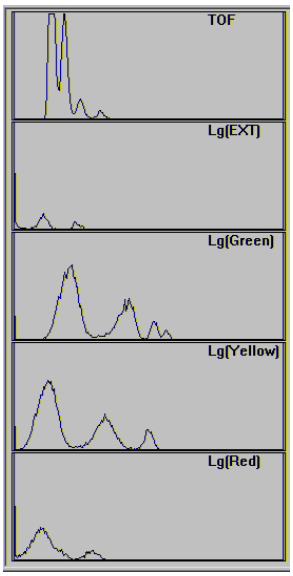
On a logarithmic scale chart, the spacing between two points corresponds to the percentage change between those numbers. Thus on a log scale chart, objects that are close together get spread apart and large spaces between objects get compressed.

Linear and Logarithmic Displays Example

Linear Scale





Log Scale

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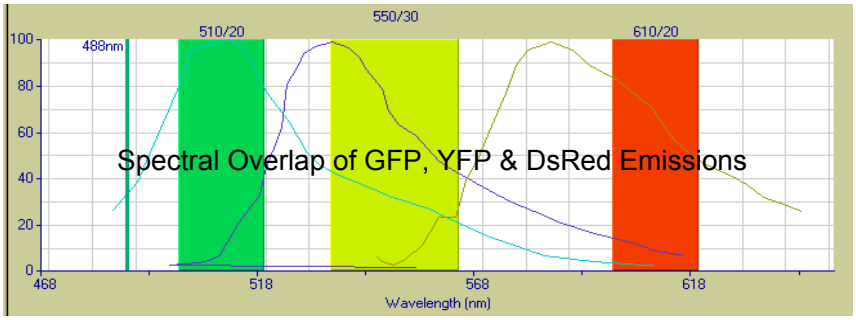
Advanced Acquisition Package (AAP) Detailed Specifications

- Fluorescence Compensation



	PMT Filters		
	Green	**Yellow**	**Red**
GFP	-	32%	3%
YFP	18%	-	9%
DsRed	0%	11%	-

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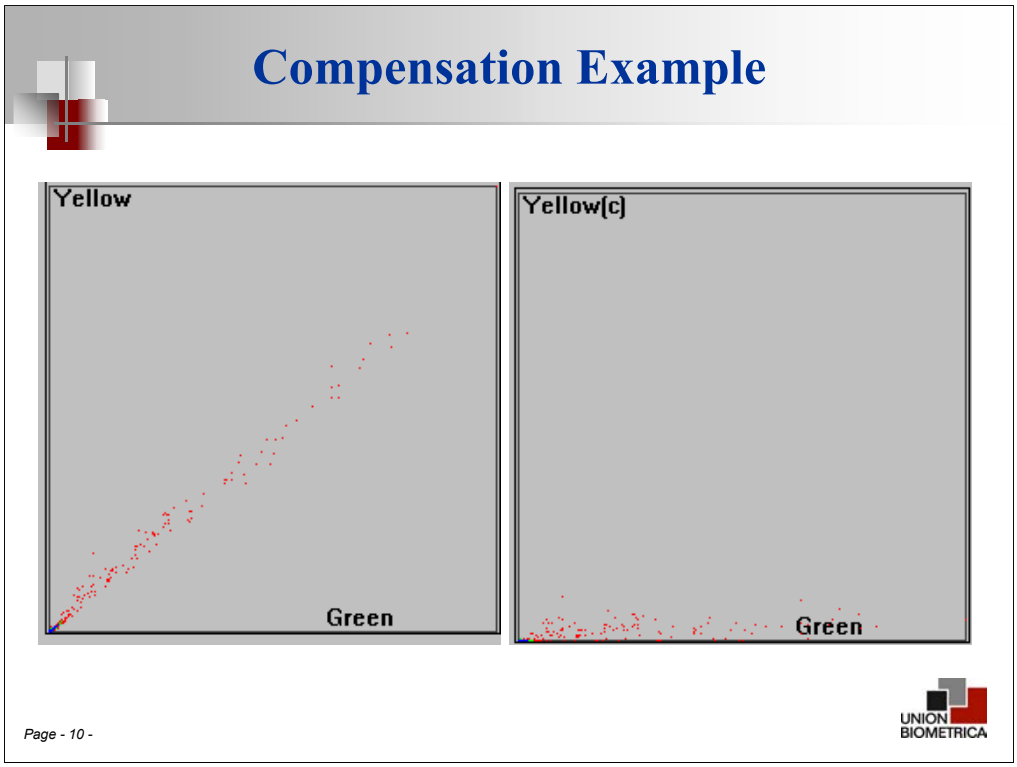
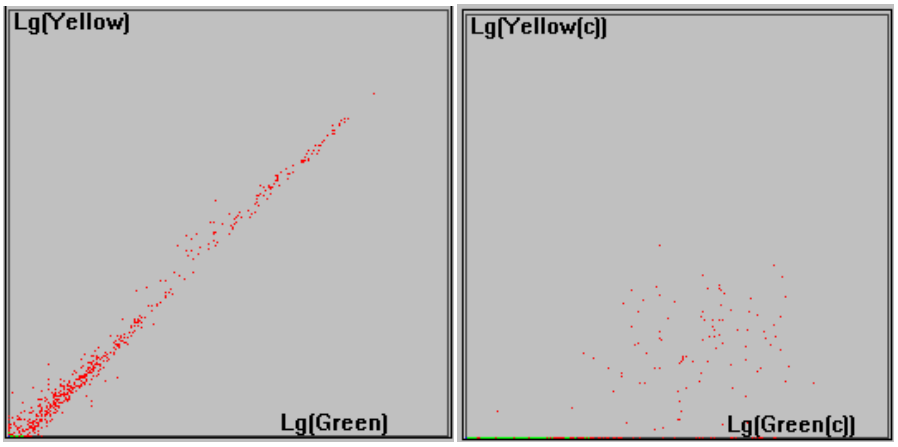
Mathematical fluorescence compensation for spectral overlap of GFP, YFP, and DsRed emissions permits detection of the presence of a weak fluorescent signal of one color even with the presence of another, stronger signal.

Fluorescence compensation is the ability to subtract the amount of fluorescence signal due to spectral overlap of GFP, YFP, and DsRed emissions from the total fluorescence signal detected for each fluorescence parameter in real time.



These dot plot screen captures display a population of *C.elegans* run on the COPAS instrument both without fluorescence compensation (left), and with fluorescence compensation in the Yellow channel (right). You are now able to detect the outliers that have come off of the axis and detect the weak yellow fluorescence independent of the green intensity (elimination of the spectral overlap).

Compensation Example 2



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These dot plots display the same *C. elegans* population as the previous slide, now run in Log Scale mode, with the right plot also with fluorescence compensation for both yellow and green. Even more so than in the previous slide, you are now able to detect the outliers that have come off of the axis due to the elimination of the spectral overlap.

Advanced Acquisition Package (AAP)
Detailed Specifications

- Parameter Math:

  $Z = aX^n$ [+,-,*,/] bY^m+c *or* $Z = a \log X$ [+,-,*,/] bY^n+c

  – Able to compensate for non-linear correlations between signals.

  – Area compensation: TOF vs. FLU (or EXT)

  $$Comp.\ FLU = FLU - k(TOF)^2 + c$$

  Note: this area equation applies only to round objects

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Mathematical Functions: The ability to perform and display on a dot plot the result of applying mathematical functions to data parameters in real time.

Introduction to Profiler II Option

- Profiler II provides simultaneous recording of 4 optical parameters (three fluorescence, one extinction), resulting in the ability to detect and graphically display variations in optical intensity along the length of an object.
  - Includes Advanced Acquisition Package
  - Up to 8000 points of data per object per channel recorded
  - Independent channel scaling
  - Extended sorting abilities with user definable sorting regions including profile peak heights, widths, locations, and numbers for each channel
  - Save and review profiles with the Profiler Reader
  - Optimize gain and PMT settings
  - Profiler I has now been discontinued

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A *Profile* is a way of analyzing individual objects detected by the COPAS by slicing each object into many thin sections along the length of the object. Each thin section is then assigned a numerical value that corresponds to the optical intensity of that section as detected by each of the Profiler channels (Ext, FLU1, FLU2 and FLU3). The result is the ability to detect and graphically display variations in optical intensity along the length of each object.

Comparison of Profiler I vs. II

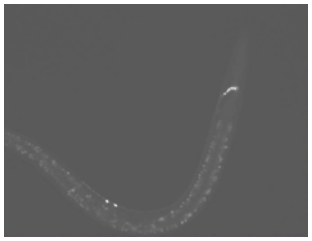
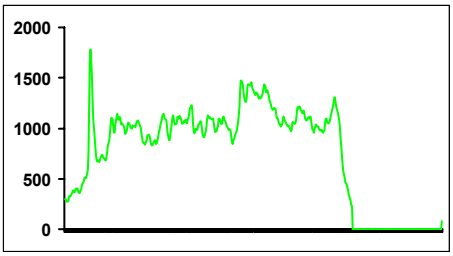
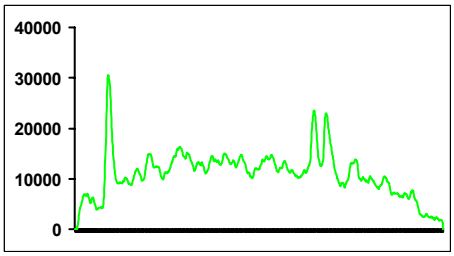


Image of *C. elegans* jsIS821 strain with GFP expressed in a cluster of synapses in the nerve ring and a pair of synaptic puncta in the ventral nerve cord.

(C.elegans strain and image courtesy of Michael L. Nonet, Washington University School of Medicine)



Profiler I



Profiler II

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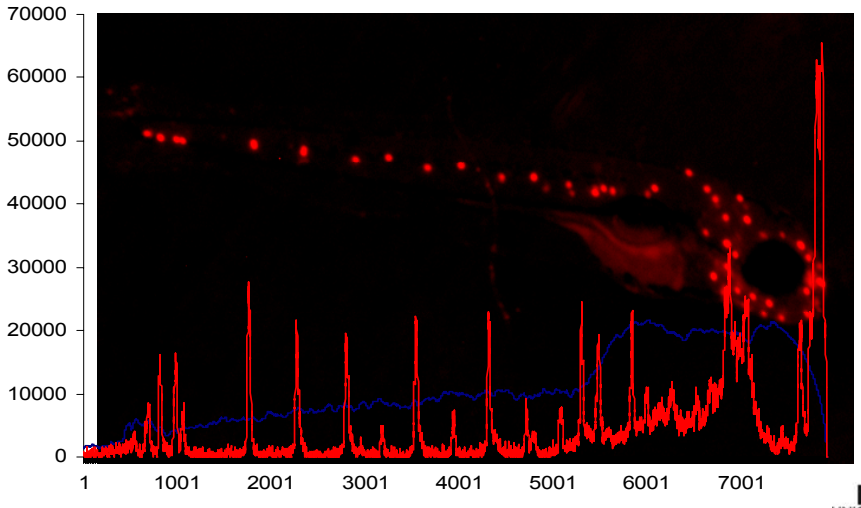
Our applications specialist, Dr. Bo Wang, tested the *C. elegans* jsIS821 strain, on the COPAS BIOSORT with both Profiler I and Profiler II and found Profiler II did indeed produce a better fluorescence profile due to the faster scanning rate and better sensitivities.

Zebrafish Image and Profile Overlay

Image of wild type Zebrafish larvae (four days after fertilization at 28.5ºC) stained with FM 1-43 with overlay of profile obtained by Profiler II.



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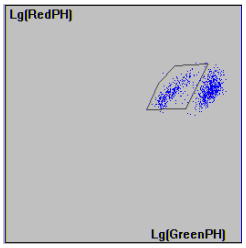
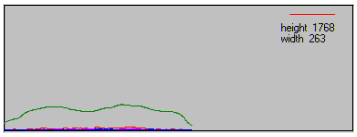
Wild type Zebrafish larvae (four days after fertilization at 28.5ºC) stained with FM 1-43 were analyzed by Profiler II and sorted individually into a 96-well plate. The images of four fish were taken and matched with their corresponding profiles. As can be seen from the above graphs, peaks of a profile match with the location of the staining spots on a fish. However, the fluorescence intensity of stained spots found in the images do not necessarily match the corresponding peak heights, possibly due to the different orientation the fish take while it is either in the flow cell or under a microscope.

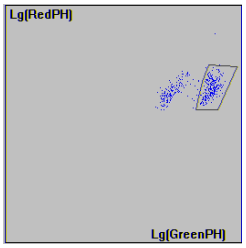
Sorting Using Peak Height

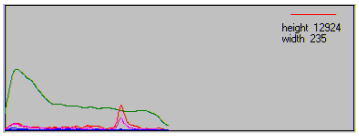
Mixed population of *C.elegans* str-1::GFP and wildtype





Profile of selected population (wildtype) *C. elegans* with no fluorescence.





Profile of selected population (str-1::GFP) *C. elegans* with fluorescence.

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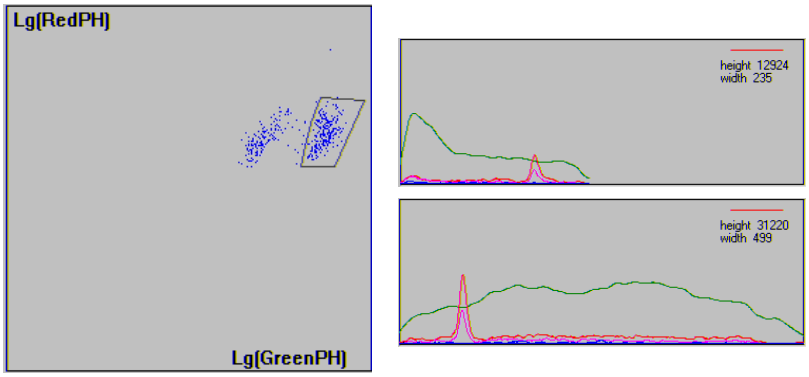
C. elegans str-1::GFP strain (GFP expression in a single neuron pair in the head and dendrites) run on a COPAS BIOSORT with Profiler II and Advanced Acquisition Package. These images resulted from selecting the larger worms (L3 stage and older) from a mixed stage population of str-1 and N2 (wildtype). Sorting on either population (using liberal regions) of GFP+ or GFP- was 97% accurate.

Sorting Using Peak Height

Mixed population of *C.elegans* str-1::GFP and wildtype



Profiles of two worms sorted from the selected bright region shown in the dot plot. Although the worm profiled in the upper graph is nearly half the size (age) and subsequently has a fluorescent peak approximately half the height of the lower graph, Profiler II still was able to distinguish this worm.

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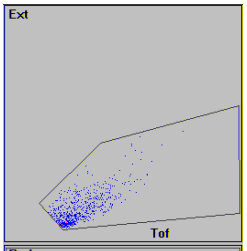
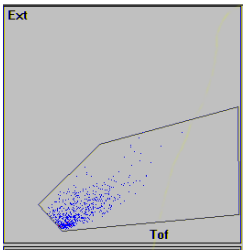
Using same mixed *C. elegans* population as previous slide, Profiler II software and the new parameters that are measured can now identify distinct populations of fluorescent and non-fluorescent worms regardless of size, therefore there is no need to stage (synchronize) the population!

Comparison of COPAS & AAP using *C.elegans* Data

Mixed population of C.elegans str-1::GFP and wildtype

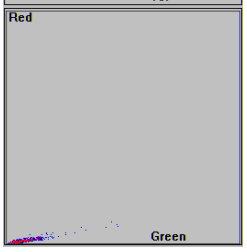
Dot plots from COPAS software.

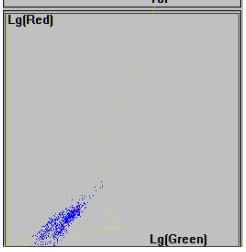




Dot plots from COPAS software with log scale feature used for fluorescence.

Although two populations are present (only one fluorescent), due to the low level of GFP expression in str-1, the standard COPAS software does not detect the fluorescence and it appears to be a single population.





By using the log scaling feature for the fluorescence measurements, more detail is shown on the dot plot, indicating the possibility of the presence of two distinct populations

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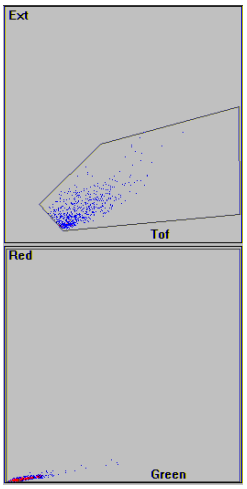
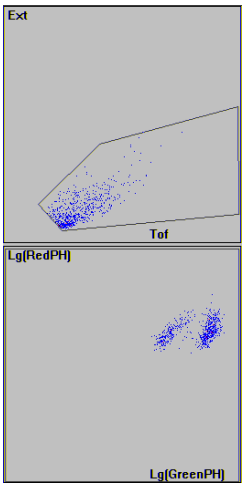
Using same mixed *C. elegans* population as slide 16, we compare analysis and sorting capabilities between standard COPAS software view versus COPAS software with Advanced Acquisition Package.

Comparison of COPAS & Profiler II using *C.elegans* Data

Mixed population of C.elegans str-1::GFP and wildtype

Dot plots from COPAS software. (same as left side on previous slide)





Dot plots from COPAS software with log scale and peak height features for fluorescence.

With the addition of using peak height to further analyze the population (used with the log scaling feature), a greater level of sensitivity is achieved and you can now clearly distinguish the two populations.

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Using same mixed *C. elegans* population as slide 16, we compare analysis and sorting capabilities between standard COPAS software view versus COPAS software with Profiler II (which comes standard with Advanced Acquisition Package).