Flow Cytometry for "Large Objects" (40-1,000 microns)

HTS of bead-based combi-chem libraries, model organisms (*C. elegans*, *Drosophila*, zebrafish), *Arabidopsis* seeds, pollen, etc.

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1. Abstract

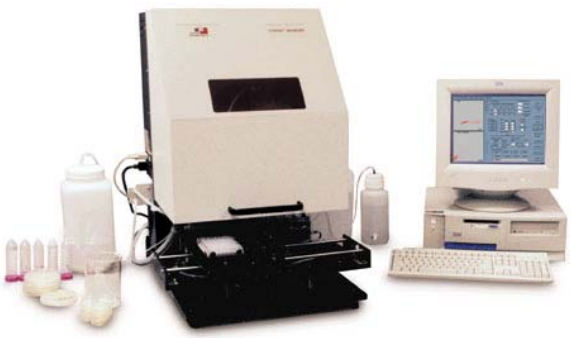
Many objects are too large and too fragile for conventional flow cytometry. Manual microscopic manipulation of model organisms is tedious, subjective, and limits the size and scope of experiments. Similarly, "bead pickers" offer only one-at-a-time manipulation of combinatorial libraries.

Instruments are now available to automate this process of sorting large (40-1,000 micron) particles in a continuously flowing stream at a rate of 10-30 objects/second. Using object size, optical density, and intensity of fluorescent markers as sorting criteria, selected beads or animals can be dispensed in multi-well plates for further validation of hits. A gentle pneumatic sorting mechanism located after the flow cell avoids harming or changing sensitive objects, thereby making the instrument suitable for live biological materials or sensitive chemistries. Multiple fluorescence excitation and emission wavelengths are available, with sensitivity for GFP, YFP, DsRed, as well as the commercially available fluorophores commonly used in combi-chem.

Beads can be quickly screened before the assay step to eliminate complications due to broken beads, bead aggregates or beads with auto-fluorescence. Binding assays, for example, can be shrunk to a single-bead, thereby minimizing the amount of precious target proteins/peptides and other expensive reagents required for a screen. Use of high-capacity 50-500 micron beads leaves sufficient residual chemistry on a single bead for *post facto* NMR and MS structure determinations.

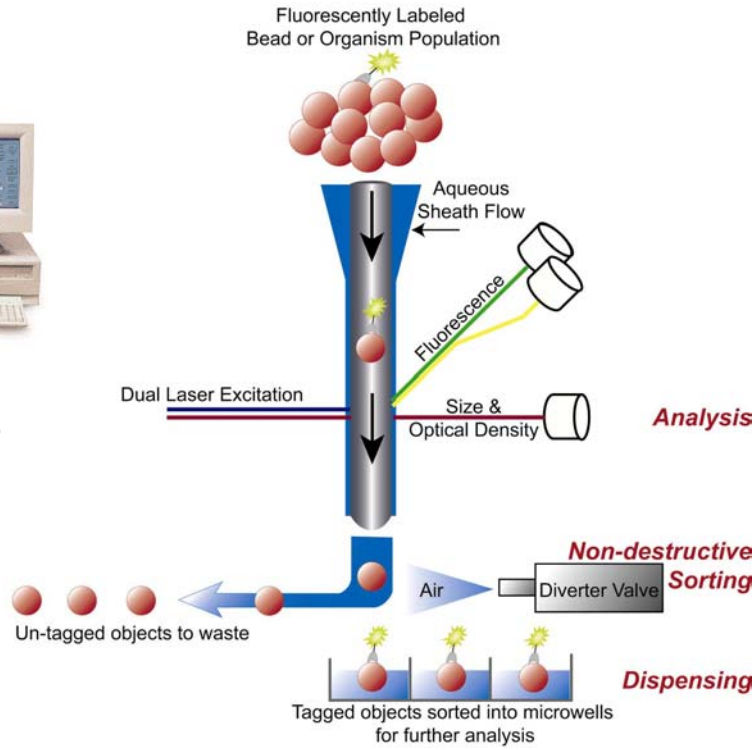
Today, automation, increased sensitivity and quantitative measurements, enable larger / faster screens of both model organisms and combinatorial libraries. Several examples will be discussed.

2. COPAS Flow Sorter



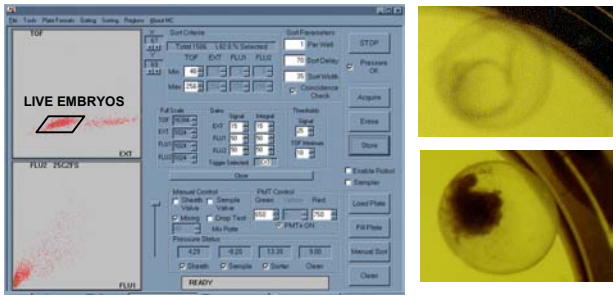
COPAS instrument shown above with standard PC, operating software, and accessories.

3. Technology Principle



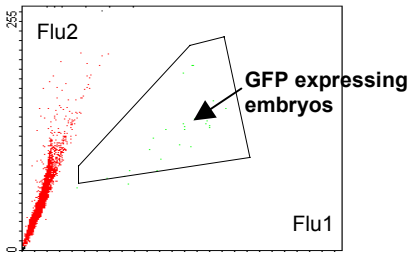
4. Model Organism Applications

ANALYZE, SORT USING OBJECT SIZE vs. OPTICAL DENSITY



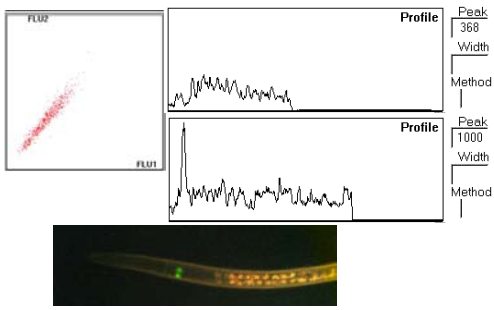
Live *D. rerio* embryos were detected and sorted from a mixed population using the COPAS XL using object size and optical density as sort criteria. Shown: Screen capture of COPAS software with results; upper image of live embryo; lower image of dead embryo.

ISOLATE RARE EVENTS USING FLUORESCENT TAGS



A genetic screen for GFP expressing *Drosophila* embryos was performed using levels of fluorescence expression. Approx. 3 million embryos were screened and GFP-expressing embryos were dispensed into microwells. Over 1,200 new GFP-expressing lines were established. Before COPAS instruments, an experiment of this size and scope was previously not possible.

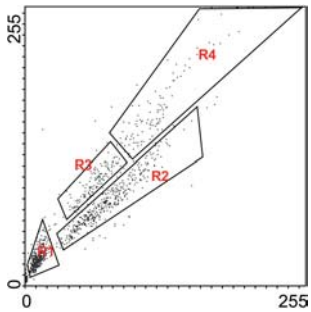
COLLECT POSITIONAL FLUORESCENCE INFORMATION



Using the Profile Module with COPAS, measure positional fluorescence along the axial length of the object to permit sorting by fluorescence peak height, peak width, number of signals, and location of signals. Shown above: dot plot showing standard COPAS total fluorescence detection capabilities, profile of a wild-type nematode, profile of transgenic nematode, and corresponding image of transgenic nematode.

5. CombiChem Applications

PRE-SCREEN SUBSTRATE BEADS FOR UNIFORM POPULATION

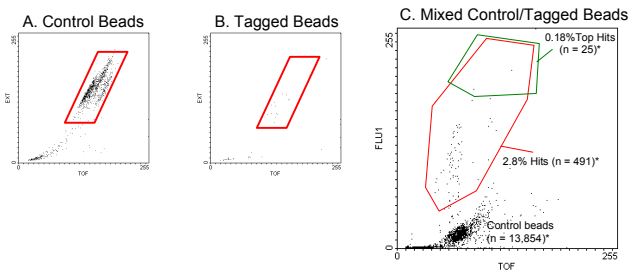


Dotplot of mixed Bead Population labeled with TxRed fluorophore
-4 distinct populations found
-Beads from these regions collected & analyzed visually fluorescence microscope

Results:
R1 region = no fluorescence
R2 region = dim fluorescence
R3 & R4 regions = strong fluorescence

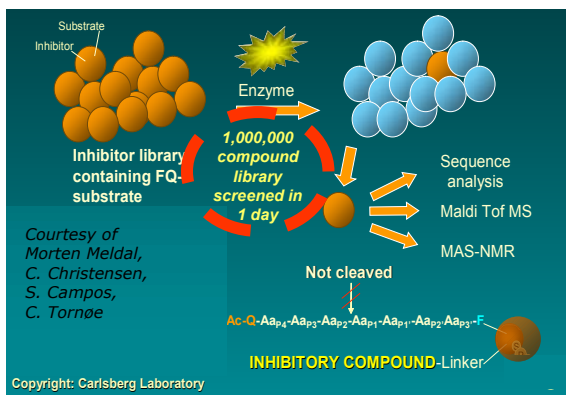
Beads may be quickly screened using object size, optical density, and fluorescence prior to the assay step to eliminate complications due to broken beads, bead aggregates, or beads with auto-fluorescence. In this case, we have been able to explain the difference in fluorescence intensity between R3 and R4 by looking at the size of the beads. R4 beads are larger, thereby accounting for the brighter fluorescence levels.

SORT HITS FROM A COMPOUND LIBRARY



By setting sort parameters, it is possible to sort strong from weak binders in a subsequent sort. As shown in the above experiment, the optical density (EXT) and size (TOF) in plots a & b show no difference in characteristics of labeled vs. control beads however, plot c shows that using bead size (TOF), the COPAS can discriminate labeled from control beads to permit specific sorting, and also that it can distinguish levels of fluorescence.

HTS OF BEAD-BASED COMBINATORIAL LIBRARIES



A one million compound Library containing a Peptide and one inhibitor candidate per bead was screened in one day (without COPAS this process to up to 2 years) and the identified 30 hits were sent for NMR / MS structure analysis of residual compound on each bead.

For more information: contact Union Biometrica, Inc.
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