UNION BIOMETRICA

COPAS™ *SELECT* INSTRUMENT
WITH ADVANCED ACQUISITION PACKAGE

Automated Analysis, Sorting, and Dispensing of small model organisms, beads, fragile large cells and cell clusters

OPERATOR'S MANUAL

Rev. 4.6
Last Updated On: August 18, 2009

DECLARATION OF CONFORMITY

Application of Conformity

73/23/EEC
And
89/336/EEC

Standards to which Conformity is Declared

EN 61010-1 (1993), Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use – General Requirements; Including Amendment Two (1995)
And
EN 61326-1:1997 + A1: 1998 Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements

Emissions: CISPR 16-1:1993, 16-2:1996 Class A
Operation: Continuous Unmonitored Operation
Location: Industrial Location

Manufacturer's Name	Union Biometrica, Inc.
Manufacturer's Address	84 October Hill Road Holliston, MA 01746
Type of Equipment	COPAS System
Model Number	COPAS *SELECT*
Serial Number	
Year of Manufacture	Serial Number Contains Date

I, the undersigned, hereby declare that the equipment specified above conforms to the above-identified standard(s).

David Strack, Ph.D., President
Union Biometrica, Inc.

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1 INTRODUCTION

1.1 COPAS™ OVERVIEW

The COPAS line of instruments from Union Biometrica, Inc. automates the analysis, sorting, and dispensing of "large" objects such as large cells or cell clusters, beads, seeds, and small model organisms using the physical parameters of object length, optical density, and the intensity of fluorescent markers. Once analyzed, objects are sorted according to user selectable criteria, and then may be dispensed into stationary bulk receptacles or multiwell (microtiter) plates for high throughput screening. The COPAS instruments have been proven to analyze and sort large objects with a higher speed and precision than present manual techniques. By automating the process, the time required for experiments is dramatically reduced, human error is eliminated, and new experiments that previously could not be considered are now possible.

The COPAS platforms handle objects ranging from 20 - 1,500 microns with four (4) different models, each featuring a specially engineered fluidic path and flow cell optimized for a subset of the size range. Various modules are also available for use with each of the models to provide additional capabilities to your COPAS system. The four models are:

- COPAS *BIOSORT*
- COPAS *SELECT*
- COPAS *PLUS*
- COPAS *XL*

1.2 SORTING PARAMETERS

Five parameters are recorded for each detected object:

- Optical density of the detected object (optical extinction)
- Axial length of the object (size)
- Simultaneous detection of three of the available three-colors of fluorescence

COPAS instruments allow for multiple fluorescence excitation and emission wavelengths. In the standard configuration, the instrument has fluorescence detectors for the green, yellow, and red regions of the spectrum to cover GFP, YFP, and DsRed™ fluorescent proteins, as well as numerous other commercially available fluorophores.

Sorting rates vary with the concentration of the sample and percentage of the total sample that is being dispensed, which is further explained in later sections within this Operator's Manual.

1.3 TECHNOLOGY PLATFORM

While COPAS instruments are designed on the basic principles of flow cytometry, the COPAS platform differs from traditional flow cytometers optimized for high-speed cell analysis and sorting in two important areas to permit larger objects to be analyzed:

- The large-bore fluidics and flow cell design permits handling objects as large as 20-1,500 microns.
- The heart of the COPAS technology is a gentle pneumatic sorting mechanism located downstream of the flow cell that dispenses objects in a fluid drop with minimal harm or change to the objects, and is therefore safe even for the collection of live biological materials or sensitive chemistries.

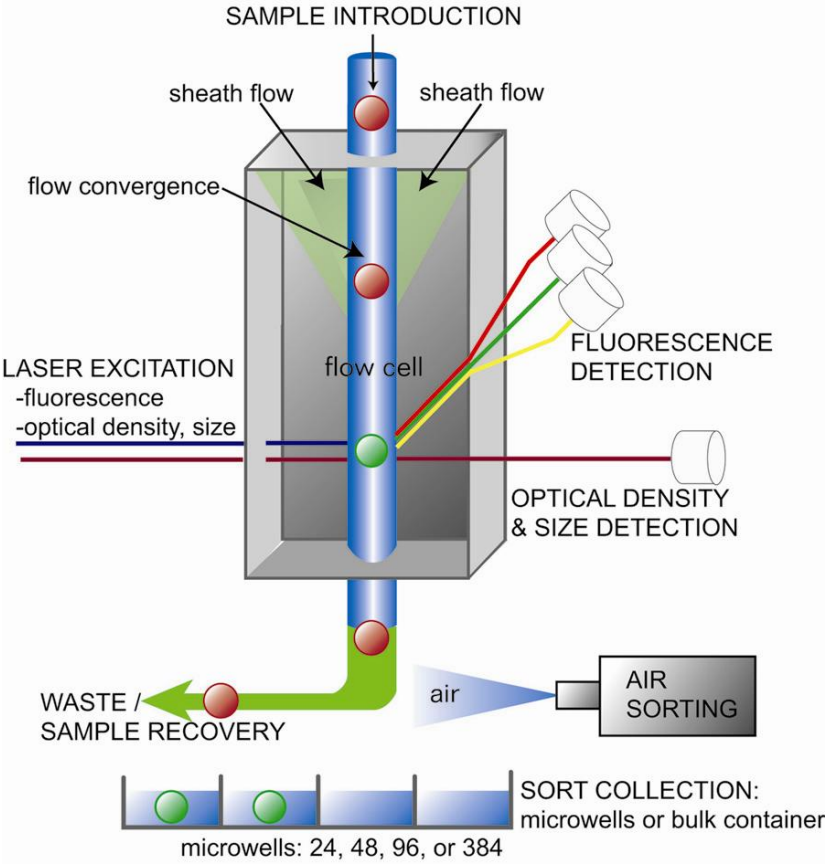


Figure 1.1: Schematic showing the principles of analysis and sorting on the COPAS instrument.

A fluid stream of sample objects flows from a continuously mixed sample cup to the flow cell. There it is surrounded by a "sheath" solution to produce a stabilized laminar flow that focuses the objects in the center of the flow stream. Objects then pass into the flow cell, where they are illuminated by at least one laser. Either a single solid state laser or a combination of laser is used to measure the axial length (Time of Flight), the optical density (Extinction) and to excite any fluorophores present. Based on the measured optical parameters (size, optical density, and fluorescence) the operator can then set gated regions for sorting and collecting the population of interest into multiwell plates or stationary receptacles.

1.4 SOFTWARE

An PC computer, such as an IBM compatible, preloaded with the COPAS software is provided with every system to provide a dedicated workstation for the analysis, sorting, and dispensing processes. Users may then transport data or access data over an existing network for further analysis.
Researchers may create, store, and retrieve specific assay files and experimental results using the COPAS software.

1.5 DATA OUTPUT AND ARCHIVING

Raw data collected from the analysis is stored as a text file (tab delineated) and in a format compatible with most flow cytometry software (list mode format), such as WinMDI or FCS Express. The COPAS software interfaces with common industry analysis tools to allow further investigation of collected raw data. The numerical raw data can be easily imported into various programs supporting graphing capabilities and statistical analysis of the sample data. The data is also stored in a proprietary file format that allows reanalysis using the COPAS user interface (bsrt file).

1.6 AUTOMATION

Instruments are equipped with an X-Y stage and software that can control the dispensing of objects into wells, as well as the ability to define various loading templates. A choice of multiwell plate formats is offered including 24, 48, and 96-well plate formats. Throughput may be further increased with the addition of a robotic-arm for plate handling, specifically for loading and unloading multiwell plates onto the system stage.

1.7 CONTACT INFORMATION

Union Biometrica, Inc. has offices in the United States and Europe.

US - Global Headquarters

Union Biometrica, Inc.
84 October Hill Road
Holliston, MA 01746
Tel: 508-893-3115
Fax: 508-893-8044
Email: sales@unionbio.com

European Office

Union Biometrica GMBH
Cipalstraat 3
B-2440 Geel, BELGIUM
Tel: +32 14 570 628
Fax: +32 14 570 629
Email: sales@unionbio.com

1.8 TRADEMARK INFORMATION:

COPAS ™ is a registered trademark of Union Biometrica, Inc.

2 SAFETY

The COPAS System is comprised of the Dispenser and the External Laser System(s). This term is used in reference to the combination of these two components.

The COPAS should be used only by trained laboratory personnel. Use of this instrument by an untrained operator could result in damage to the product or injury to the operator.

Other than procedures discussed in this manual, there are no other operator required electrical, optical, or mechanical adjustment or serviceable components.

Use of the COPAS Dispenser and External Laser System(s) in a manner not specified by Union Biometrica may impair the protection provided by the equipment.

2.1 SYMBOLS AND NOTES USED IN THIS OPERATOR'S MANUAL

This symbol throughout this manual indicates to the user that they should take careful note of the information presented and thoroughly read the section prior to operating the COPAS instrument.

NOTE: This term is used throughout this document to emphasize important operating information or important information for the operator.

Bold type: This type of font is used throughout this document to highlight important operator information.

ALL CAPS: This type refers to a menu item in the COPAS Software screen

2.2 SAFETY HAZARDS

No flammable liquids that can cause the spread of fire in normal conditions or in single fault conditions are contained in, or specified for use, with this instrument.

No hazardous substances are required or specified for use with this equipment, and no hazardous substances are produced by its use.

Any reagents provided by Union Biometrica, Inc. for use with this equipment are shipped with the appropriate Material Safety Data Sheet (MSDS) for safety related information.

If any chemicals not supplied by Union Biometrica, Inc. are used in conjunction with this instrument, be certain to observe safe laboratory practices and refer to the appropriate Material Safety Data Sheet (MSDS) for safety related instructions.

If the equipment is not used in a manner specified by the manufacturer, the protection provided by the equipment may be impaired.

Refer to the Requirements (Section 9), Installation (Section 11) and Specifications (Section 12) chapters of this manual regarding use of the removable line cords as emergency disconnect devices. Refer to the Warning Labels section below and understand the hazards that are being referenced by the labels.

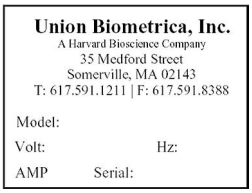
In order to prevent the system from overheating, insure that the installation permits the unrestricted flow of air around the system components.

2.3 SAFETY SYMBOLS LOCATED ON THE COPAS INSTRUMENT

This label located on the COPAS system indicates to the user that before using the COPAS and External Laser System(s), they should carefully read this Operator's Manual. Do not remove any part of the system labeled with this symbol; injury or damage to the system may result.

This label located on the COPAS system indicates a pinch hazard where users should take care not to place hands or other objects in the hazard area in order to avoid injury or damage to the system.

2.4 GENERAL LABELS LOCATED ON THE COPAS INSTRUMENT



Located next to the main power switch, this label indicates the manufacturer (Union Biometrica, Inc.) address, and fax information as well as the COPAS instrument information: Model, Volt, Hz, AMP, and unique instrument Serial Number.

Located next to the main power switch, this label indicates the "ON" position.

100-240V ~ 50-60 Hz 120W
Fuses: 2.5A 250V Type M

Located next to the main power switch, this label specifies the COPAS instrument fuse ratings.

SERIAL

This label indicates where the serial communications (RS-232) cable input is connected on the instrument

LASER CONTROL

This label can be found in several locations, on the instrument next to the connection for the fiber-optic cable input each laser; and next to the fiber-optic cable input on each laser head.

PRESSURIZED AIR

Located next to the Pressure Regulator on the instrument, this label indicates to users that this input is under pressure.

RJ-45

Located on the rear of the computer to indicate where the Profiler option input is located.

A IOIOI

Original manufacturer's mark on the computer rear panel, international symbol indicates COM1 Serial port.

Original manufacturer's mark on the computer rear panel, international symbol indicates USB port.

3 COPAS *SELECT* DESCRIPTION

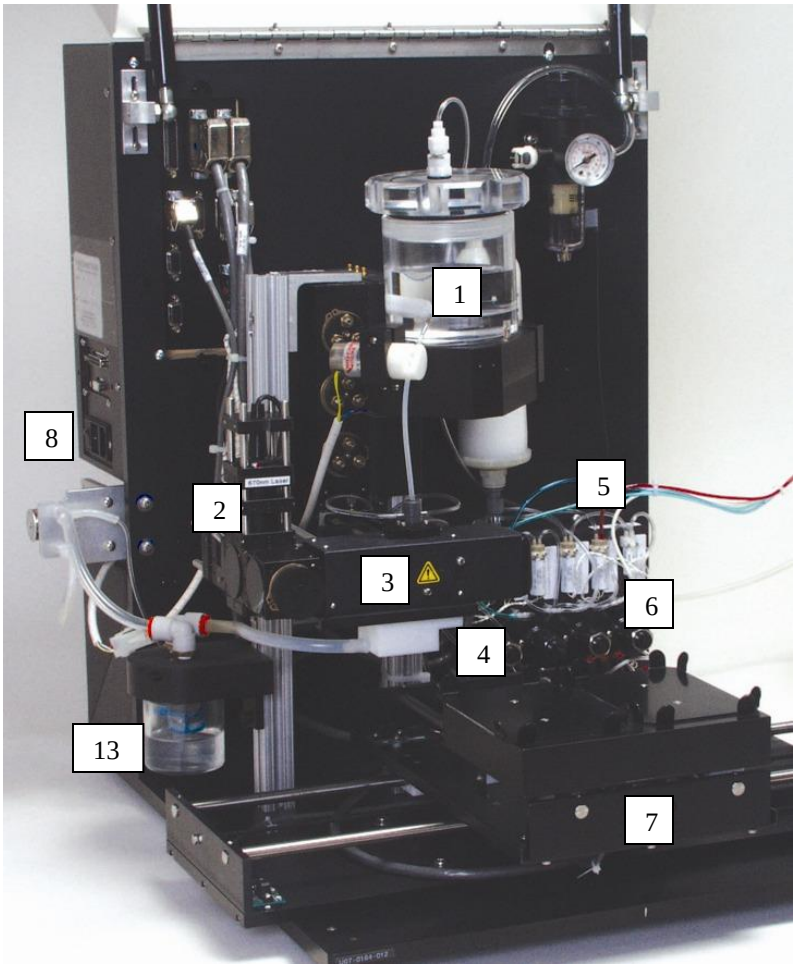


Figure 3.1: Operator's view of COPAS instrument.

3.1 DESCRIPTION OF COMPONENTS

1) Sample Cup:

The sample cup is located above the optics assembly. The standard *SELECT* comes with a 250 ml sample cup. An optional 40 ml sample cup is available for purchase. One stir bar continuously mixes the sample to prevent sample sedimentation from occurring at the bottom of the cup. This ensures correct introduction of the sample into the flow cell.

2) Laser Optics Assembly

The optics assembly consists of at least one laser focused on a quartz flow cell in one of the following configurations.

2 laser system utilizing a red diode laser (635 or 670nm) for size and optical desity determination and a fiber optic connection from the external multi-line laser wiith a changeable filter used to select excitation wavelengths of 488 or 514 nm. This excitation filter is located directly in front of the fiber connection. Make sure the arrow on the filter points away from the laser source.

A single/multiple solid state laser system utilizing a 488nm laser alone or in combination with other solid stare lasers such as 405nm, 561nm, or 640nm emissions.

	A photon electric sensor is located on the laser beam axis. Orthogonal emitted light is collected by PMTs (photo-multiplier tubes).
3) Flow Channel	The flow channel is a quartz assembly containing a 500 µm channel through which the sample flows. The sheath fluid is forced through this channel, producing a stabilized laminar flow condition. The sample is "pushed" into the flow and focused to the center of the flow stream. The speed of the sample flow orients the objects into a single file, straight configuration.
4) Diverter (Sorter) Valve	The diverter valve sorts the object selected by the user. The diverter valve is set to the ON position normally and deflects the fluid stream at approximately a 35º angle into the waste tray. When an object meeting selection criteria is detected in the flow cell, a message is sent to the diverter valve to turn OFF and then ON again, to generate a droplet of liquid containing the sorted object, thereby permitting the object to drop into a collection device.
5) Valve Pressure Panel	The four valve regulators adjust the pressures for the following: the sheath reagent, the sample, the Clean reagent (via back flush), and the air diverter. Initial pressures are factory set. Certain instrument and sample conditions may require adjustment of these pressures for sorting efficiency.
6) Pressure Regulator	Pressure is supplied by an external compressor and is regulated for the system at approximately 25-30 PSI. The pressure regulator allows adjustment and monitoring of the pressure.
7) X-Y Stage	The stage supports the positioning of microtiter plates or other collection devices. Alignment of the stage is required for optimal sample collection. The left side of the stage is referred to as Position A and the right side is referred to as Position B.
8) Power Switch	The power switch is located at the rear on the left side of the system.
9) Sheath Container	The sheath container is filled with an appropriate Sheath Reagent fluid. The 10 liter sheath container supplied with the standard *SELECT* should be filled with 10 liters of the appropriate Sheath Reagent and refilled before the level drops to below about 500 mls. A full container of sheath reagent will run for approximately 6 hours of continuous use (not shown in picture).
10) Waste Container	The waste container is supplied with the instrument and holds either 4 liters or 10 liters. The contents should be removed and disposed of each time the sheath container is filled (not shown in picture).
11) Cleanout Bottle	The Cleanout bottle contains the same liquid as the sheath fluid and is used to supply a back flush during the Clean cycle. The Clean cycle is used to remove air bubbles or any other debris that may have settled during normal system operation that may cause a clog in the flow cell (not shown in picture).
12) External Laser	The multi-line Argon laser and Solid State Lasers are each a separate unit. Light is routed to the laser optics assembly by a fiber optic cable. Each laser is controlled by an ON/OFF switch and in some cased by the COPAS software.
13) Sample Recovery unit	The optional sample recovery unit collects all the sample that is not dispensed so it can be used again.

4 OPERATING THE COPAS *SELECT*

This chapter describes standard operation of the COPAS *SELECT*. Additional details and explanations of the software features can be found in section 7 COPAS SOFTWARE. It would be of value to establish a log book that allows users a place to document and track instrument performance.

Gate region dot plot

Sort region dot plot

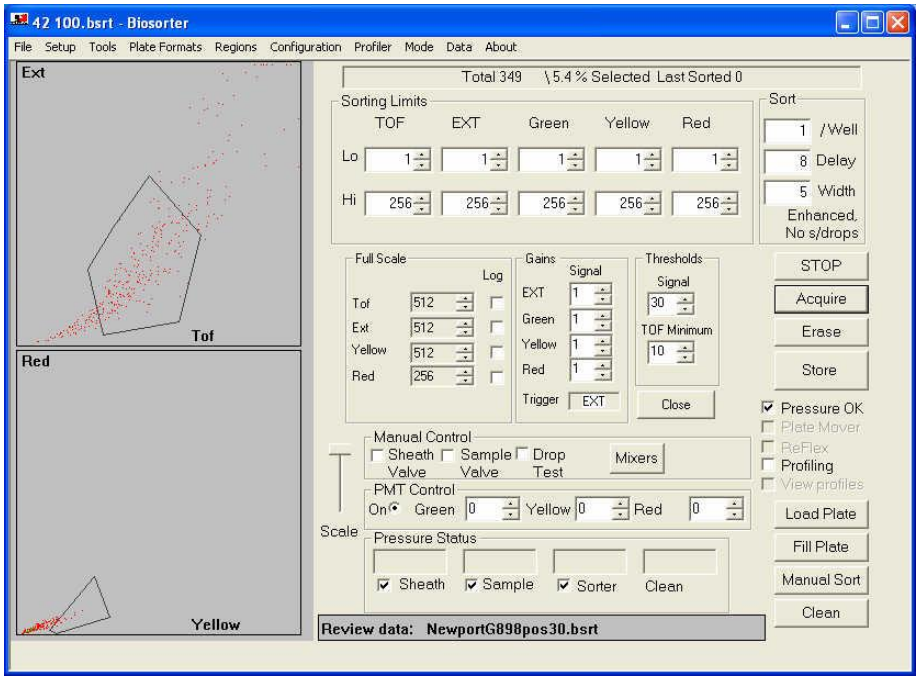


Figure 4.1: Screen print capture of COPAS software

4.1 PREPARATION

1. Turn on the main power of the COPAS *SELECT* system. Turn on the computer.
2. Turn on the compressor.
3. If using an Argon laser, ensure that the correct excitation filter is in place. Turn on the main power of the external laser (by pressing the GREEN button).

> ***TIP!***
> *The two choices for excitation filters are 488 nm and 514 nm when using the Argon laser.*

4. Check the waste container and sample recovery bottle and discard any waste contents that may have been left in the container from previous operations.
5. Fill the sheath container and firmly tighten the cap. Refer to section 10 REAGENTS, for information on optimal reagent usage for your application.
6. Fill the Cleanout bottle with sheath reagent and firmly tighten the cap. Attach the Cleanout bottle to the appropriate fitting.
7. Select the Union Biometrica software icon on your PC using the computer mouse. Double click the icon to open the software.
8. Click the START button in the COPAS software main screen to supply pressure to the sheath and sample containers.
9. The External Laser Control pop up window will appear. Select RUN mode and wait as the laser powers up.
10. When laser power reaches approximately 10 ± 1 mW, select DONE on the External Laser Control pop up window.

11. When pressures have equilibrated to previously set levels, click the PRESSURE OK check box on the main software screen to launch the priming sequence for the preanalysis chamber and flow cell.
12. Click the Clean button. This sends a high pressure stream of fluid from the Clean bottle into the preanalysis chamber and flow cell dislodging any bubbles and/or debris from the flow cell and sample tubing. If debris is dislodged into the sample cup, the sample cup should be cleaned by following the Sample Cup cleaning procedure using bleach and cleaning solution (Section 6 MAINTENANCE).
13. Check the sheath reagent flow rate. To do this, open the sheath valve by putting a check mark in the box by the Sheath Valve in the Manual Control box. Then hold a graduated 50 ml tube directly below the opening in the waste tray used for dispensing. Manually turn off the diverter pressure by clicking on the check mark in the box beside the label Sorter, near the bottom of the software screen (below the Pressure Status fields).

TIP!
Typical Sheath Pressure at ambient temperature (22°C) is in the range of 3.5 to 5.0 PSI. Sheath Pressure should allow for collection of 25 ml of sheath fluid through the flow cell per minute. Specific setting determined at factory and by Field Service Technician at time of installation.

Typical Sample Pressure at ambient temperature (22°C) is in the range 1.9 to 3.0 PSI. Specific setting determined at factory and by Field Service Technician at time of installation.

14. Collect sheath reagent for 1 minute and then turn the divertor valve back on by checking the box beside the label 'Sorter', near the bottom of the software screen. The amount of sheath collected in 1 minute should be between 22-25mls. If necessary, adjust the sheath pressure by changing the tension of the sheath regulator via the center screw. Allow one minute for the pressures to equilibrate and test volume output again, until the value remains stable and sheath output is between 22-25mls per minute.

CAUTION: If using an Argon Laser, make sure the proper excitation filter is installed and that the arrow on the filter is pointing away from the laser source.

CAUTION: During instrument set up and sorting, the X-Y stage moves to align and accept the sorted sample into the appropriate well. Care should be taken not to place fingers or lab equipment on the stage, injury and/or damage to the instrument could result.

4.2 RUNNING CONTROL PARTICLES

NOTE: Union Biometrica, Inc. recommends processing control particles daily while instrument is in use.

NOTE: Each instrument's settings may differ from the generic settings below. Please refer to the screen printout of the control particle results that were run during installation or at the last preventative maintenance visit. This screen print will indicate the proper pressures and PMT voltage settings for your instrument.

1. Prepare the COPAS *SELECT* instrument as instructed in 4.1 PREPARATION
2. Mix appropriate high fluorescence control particles by inversion 4 times. Do not shake. Avoid any excess agitation that may cause bubbles in the mixture. Refer to section 10 REAGENTS for information on optimal reagent usage for your application.

3. Place a minimum of 20 ml of the control particles into the sample cup. Use the COPAS ESS High Fluorescent Control Particles (Union Biometrica P/N 335-5071-000) if you using ESS Sheath reagent (335-5070-000). Use the GP Control Particles Hi-Fluorescence (310-5071-000) if you are using GP General Purpose Sheath. .
4. Open the TOOLS drop down menu and select RUN CONTROL PARTICLES.
5. Identify PMT settings previously used and input those settings for the Green, Yellow and Red PMTs. These are located under the PMT Control heading on the main COPAS *SELECT* software screen.

NOTE: THRESHOLD, GAINS AND SCALE values are preset while in RUN CONTROL PARTICLES mode.

6. Press the CLEAN button to remove any debris or air from the flow cell. It may be necessary to use the CLEAN button two to three times in order to remove all bubbles from the system.
7. Click on the ACQUIRE button to begin sheath and sample flow and allow pressures and sample stream to stabilize.
8. Document the instrument's pressures found in the pressure status fields on the main screen.
9. Adjust the sample pressure, slowly turning the sample valve knob, until system flow reads 5-10 objects/second.
10. Once a stable flow rate has been achieved, erase the acquired data by clicking the ERASE button and process beads until 500 objects have been acquired.

NOTE: System has BEAD SAVER mode (see bottom left corner of user screen). When selected, this feature automatically stops the flow of sample and sheath fluids after 500 beads are acquired. User may turn this off during the operation by unchecking this box.

Document the MEAN and C.V. of the particles from the top histogram titled TOF (you may prefer saving a software screen image-option found in the FILE menu). MEAN indicates the mean channel of the histogram. C.V. represents the coefficient of variation around that mean. These values are an indication of instrument performance, showing that the red diode laser is operating properly for obtaining TOF and EXT readings and also that the optics are clean and in working order. Results should fall within the following ranges:

TOF C.V.	≤ 11.0
TOF MEAN CHANNEL	40 +/- 6.0

NOTE: The MEAN CHANNEL and C.V. for TOF and EXT should remain consistent from day to day. Shifts in the Mean or C.V. of the particles may indicate problems with the instrument. (See sections 7 COPAS SOFTWARE and 13 TROUBLESHOOTING**)**

TIP!
Tracking the FLU MEAN and C.V. is important for verifying proper operation of the external laser; however it is not an indication of the instrument's performance.

4.3 FLUSHING PROCEDURE (FOR REMOVING CONTROL PARTICLES OR SAMPLE FROM FLOW PATH)

NOTE: Always Close the sheath and sample valves before opening the sample cup. If the sheath and the sample valves are left open while the sample cup is open, the sheath fluid will flush back into the sample cup.

1. Press the CLEAN button on the screen twice.
2. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
3. Detach pressure tubing from the sample cup cap and remove the cap.
4. Rinse the sample cup with deionized/distilled water.
5. Aspirate/remove off the water and fill again with deionized/distilled water. Repeat twice.

6. Cap the sample cup and re-attach the pressure tubing.
7. Select the SAMPLE VALVE check box. A warning message will appear stating that opening the sample valve will contaminate the flow cell. Click OK and process the water until the sample cup is nearly empty. Check the SAMPLE VALVE check box again to shut the valve and stop sample flow. System is ready to run samples. If sample is not to be run immediately be sure to leave a minimal amount of water in the sample cup.

4.4 RUNNING SAMPLES

4.5 NOTE: If a sterile sort is required, perform the appropriate sterilization procedure according to section **6.2 BLEACH PROCEDURE**

1. Close the SAMPLE and SHEATH VALVES by removing the chek from their checkboxes.
2. Detach the air pressure tubing and remove cap from the sample cup.
3. Discard any remaining sample.
4. Fill the sample cup with 20 ml of deionized/distilled water and firmly tighten the cap.
5. Attach air pressure tubing to the sample cap.
6. Re-check the SAMPLE VALVE checkbox. The warning message, 'Caution, this Operation will contaminate the Flow Cell if Sample is Present", will appear. Click OK.
7. Process the water until the sample cup is nearly empty.
8. Close the SAMPLE VALVE by removing the check from its checkbox.
9. Detach the air pressure tubing and remove cap from the sample cup.
10. Fill about half the sample cup with 50% bleach solution (final hypochlorite concentration should be ~2.5%), refer to section 10 REAGENTS, for bleach concentration specifications, replace the cap and firmly tighten.
11. Attach air pressure tubing to the sample cap.
12. Re-check the SAMPLE VALVE checkbox. Click OK when error message appears to process the bleach until the sample cup is nearly empty.
13. Close the SAMPLE VALVE.
14. Open the SHEATH VALVE and allow sheath to process for 3 minutes.
15. Detach the air pressure tubing and remove cap from the sample cup.
16. Rinse the sample cup twice with ~20ml of deionized/distilled water aspirating contents between rinses.
17. Fill the sample cup again with 20 ml of deionized/distilled water and firmly tighten the cap.
18. Attach air pressure tubing to the sample cap.
19. Re-check the SAMPLE VALVE checkbox. Process the water for several minutes without letting the sample cup to run dry.
20. Click STOP once.
21. Clean external surfaces of any spillage using a water dampened lint free cloth.

COMPLETE STERILIZATION PROCEDURE**.**

4.5.1 ACQUISITION

1. With SHEATH and SAMPLE VALVES closed (no check mark in Manual Control boxes), remove the cap from the sample cup and aspirate off any deionized/distilled water that may be present.

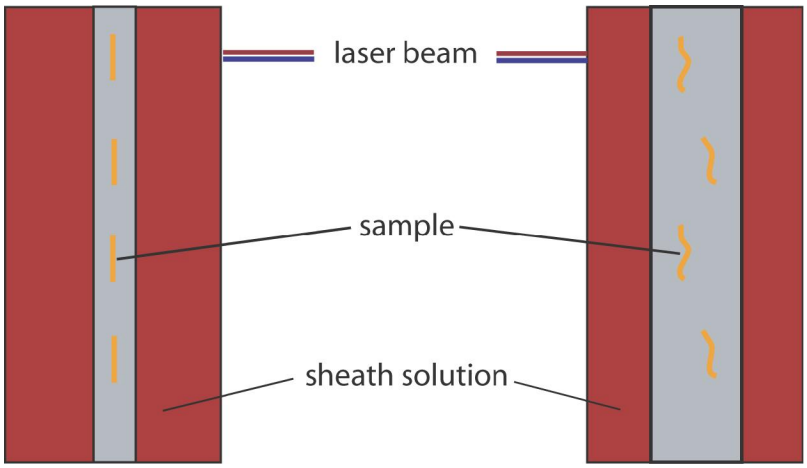
2. Fill sample cup with a minimum of 5 ml and a maximum of 40/250 ml of sample (depending on the size of the sample cup) and then re-cap the sample cup.
3. Empty and clean any contents from the sample recovery cup. Remove sample sieve, inspect for holes or other problems. Replace a new or cleaned sieve to the housing if necessary.
4. Press the CLEAN button to remove any debris or air from the flow cell.
5. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
6. Open the TOOLS drop down menu and select ADJUST GAIN SETTINGS.
7. Choose SETUP Mode under the MODE drop down menu. This will allow you to make data display and sorting option changes during instrument set up.

NOTE: SETUP mode will allow the user to make changes in sampling conditions (ie PMTs, GAIN SETTINGS, GATE and SORT PARAMETERS, and REGIONS) but data cannot be saved during SETUP mode. COLLECT DATA mode will not allow the user to make changes until acquisition is stopped. All modifiable settings will be grayed out and unchangeable during COLLECT DATA mode.

9. Click the ACQUIRE button to begin sheath and sample flow. Note there will be a delay before the sample valve is opened.
10. Allow sample to acquire for a minute to stabilize the sheath and sample flow.
11. Adjust the sample concentration, adding additional sample or diluting contents of sample cup, if necessary, to obtain stable flow of the objects (15-50 events per second).

TIP!
The objective is to obtain a minimum sample pressure. A narrow sample stream is centered relative to sheath flow (Figure 4.1). This aids in maintaining consistent velocity and delay. The sample concentration is therefore important and should be checked.

Typical Sheath Pressure at ambient temperature (22°C) is in the range of 3.5-5.0 PSI.
Typical Sample Pressure at ambient temperature (22°C) is in the range of 1.9 -3.0 PSI.



Correct Object Alignment

Correct alignment of the objects using low sample pressure with a correct sample concentration

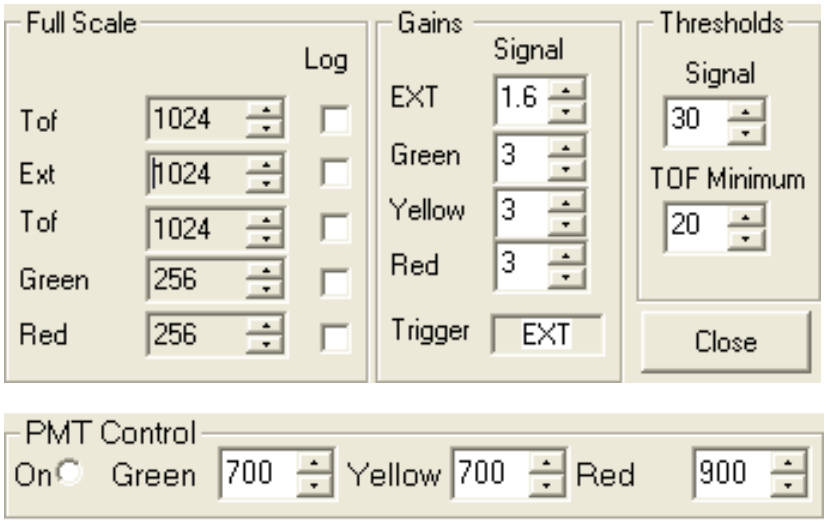
Incorrect alignment of the objects using high sample pressure with a low sample concentration.

Figure 4.2: Schematic showing the optimized core sample stream

CAUTION: Because the COPAS *SELECT* is a pressurized instrument, all reagents should be kept at ambient temperature at least 24 hours prior to use. If ambient temperature changes more than +/- 1.5ºC, adjustments may have to be made to the Sort Delay.

12. Select a stored file template by opening an existing file under the FILE drop down menu. Or adjust the instrument parameter settings (Gains, Gate and Sort dot plot parameters) for proper analysis of the sample. Adjust settings based on type and size of objects to be analyzed. Detailed descriptions of the adjustable settings can be found in sections 7.6 PARAMETERS FOR ANALYSIS and 7.7 ADJUSTING GAIN VALUES.
13. Adjust PMTs to allow appropriate amplification of the fluorescence signal. Different samples will require different PMT settings. When the PMT voltage is set to 0, the PMT is off. Maximum PMT value is 1100. Gain values range from 1 and 1100 and are non-linear. Typical operating zone between is in the 200-900 range.

THRESHOLD, GAINS AND SCALE RECOMMENDATIONS



14. Select appropriate parameters for analysis. In the HISTOGRAM mode, drag the vertical lines located at the left and right boundaries of a single histogram or combination of histograms to mark the data points of interest. Or double click on the histogram to obtain dot plot display of data points. In DOT PLOT mode, select parameters for dot plot display using the GATE and SORT dot plots under the CONFIGURATION pull down menu. Then draw Gate and Sort regions using options in the REGIONS pull down menu to include data points of interest. For a detailed description of markers, gate, and sort regions refer to section 7.11 SORT CRITERIA DISPLAY.
15. Click the PAUSE button once to stop data acquisition and then the sheath, and sample valve check boxes to stop the flow.
16. At this point if you wish to acquire data for storage, select COLLECT DATA, under MODE pull down menu.
17. Erase previous data.
18. Click ACQUIRE button to begin acquisition for data collection.
19. Once data is collected, click the PAUSE button once to stop data acquisition and then the sheath, and sample valve check boxes to stop the flow.

20. Click the STORE button to save the data. A dialogue box opens, prompting the user to choose a directory and file name to save acquired data. Upon clicking OK, four files are automatically generated including: a txt file (raw data), lmd file (configured for flow cytometry analysis, also called list mode), a bsrt file for reanalysis in the COPAS Software and a csv file (COPAS system template). See DATA STORAGE, Section 8, for more information on data storage.
21. Analyzed objects will be retained in the sample recovery bottle. Contents can be transferred to another receptacle for storage or reuse.

4.6 SORTING (DISPENSING THE SAMPLE)

For further SORT details and instructions, refer to sections 7.11 SORT CRITERIA DISPLAY and 7.13 SORT PARAMETERS.

4.6.1 PREPARATION

2. Select a collection device from the PLATE FORMATS drop down menu: well options are 24, 48, and 96. If collection will occur into a bulk collection device, refer to section 4.9 MANUAL SORTING.
3. Alternatively, click on the CLOSE button located to the left of the STORE button. This will close the view of the signal gains and thresholds, making visible the SORT GRID AREA. Define which wells and the order to be filled. By clicking in a well field, for example A1, a number will appear thereby designating that well will be filled and its order in the filling sequence. This may be done in each well field. For rapid sequence selection, hold the shift key down and using the cursor, move the mouse over the desired well fields in the desired fill order.

4.6.2 SELECTING SORT CRITERIA

System can sort while screen display is in Histogram or Dot Plot mode. See section 7.11 SORT CRITERIA DISPLAY for more information how to optimize sort criteria.

1. Select TOF gate markers on the TOF parameter in the Histogram Mode (if applicable). The data outside this marked region will not be displayed in dot plots.
2. Double click in body of the histogram to switch the view to two dot plots.
3. Select the combination of parameters from the CONFIGURATION pull down menu, select GATING AND SORTING, to define both Gate (upper) and Sort (lower) dot plots independently.
4. Select Define a GATE REGION from the REGIONS drop down menu. Using the mouse, draw a polygonal shape around the population of interest in the Gate (upper) dot plot.
5. Select Define a SORT REGION from the REGIONS drop down menu. On the lower dot plot, draw a polygonal shape, using the computer mouse, around the population of interest.

NOTE: Gate and sort regions can be modified by choosing to EDIT GATE/SORT REGION under REGIONS menu. Once selected, user can change coordinates of region boundaries either by inputting new coordinates or dragging a polygon marker to a new location. Select OK when done.

4.6.3 ALIGN STAGE

1. Make sure sheath is flowing and events/well to dispense is set to 1. Select ALIGN PLATE HANDLER & STAGE from the TOOLS pull down menu.
2. Click the LOAD A button in the ALIGN PLATE HANDLER & STAGE screen and place the selected collection device on the stage.
3. Click the WELL A1 button. Then click the TEST DROP button. Verify that the test drop has been deposited cleanly into the correct well.
4. If drop is not correctly deposited, stage alignment will need to be adjusted. Locate the stage coordinate boxes above the WELL A1 button. Increase/decrease stage movements <OUT IN> and <LEFT RIGHT> accordingly. Stage movements can be made by clicking on the arrows above the <OUT IN> window to move stage forward and backward, arrows below <LEFT RIGHT> window to, move the stage left and right, or by highlighting and changing the coordinate integer inside either window. Click the WELL A1 button and repeat the TEST DROP procedures in step 3 to verify correct stage alignment.

5. Click the NEXT COL(lumn) or NEXT ROW buttons to verify additional well positions are aligned, adjusting the step value between wells for accuracy. Note: Always start from WELL A1 whenever a change has been made.
6. TEST DROP to multiple locations on plate as well as the LAST WELL location to verify correct stage alignment over the entire plate.

   Note: If using ReFLx option for C. elegans analysis both plates must be precisely aligned to allow clearance for ReFLxing probe. It may be necessary to physically move the right or left plate holder to correctly align both plates. To do this, remove the multiwell plate from the holder at position B, and loosen the screws holding the plate holder to the stage. Gently move plate holder the desired direction and loosely tighten screws on holder. Check new alignment using ALIGN STAGE procedure and repeat if necessary until both plates are in exact alignment. Once both plates are aligned firmly tighten screws holding plate holders to the stage. Alignment is correct when the test drop accurately hits well A1on a plate on Stage A and the probe of the ReFLx is positioned above well A1of a plate on Stage B.
7. Click the DONE button, which will return the view to the COPAS software main screen.
8. Save the stage alignment upon exiting the program. Click SAVE/SAVE As under FILE pull down menu. The alignment will be saved in the COPAS system software.

4.6.4 VERIFY SORTING ACCURACY

1. Select number of events to be sorted per well by entering the desired value (between 0 and 50,000) in the /WELL field located on the main software screen beneath the Sort heading. On a 96 well plate SORT GRID AREA, select the well positions in which to dispense the sample.
2. Set the SORT DELAY (amount of time delay for drop to be dispensed). Typically, under normal sample conditions, a value of about 8 is sufficient for objects to move into position to be dispensed. If the delay needs to be adjusted, increase or decrease the delay in increments of 0.5. Test dispensing some of the sample and determine if sorting has improved. Make necessary changes until optimal sort results are achieved.
3. Set the SORT WIDTH (size of sample drop). Typically the sort width of the COPAS *SELECT* is about 7. However, drop width may be smaller or larger if the sample objects are consistently very small or larger respectively. To adjust the sort width, increase or decrease the value in increments of 0.5, dispensing a few objects to verify dispensing accuracy. Continue to make changes until optimal sort results are achieved.

TIP!
The SORT DELAY setting is a function of the sheath pressure. If the sheath pressure is changed, the SORT DELAY must be verified.

4. Ensure COINCIDENCE CHECK reads enhanced or pure signifying events are checked for coincidence (whether more than one event falls within a single drop width). Change this setting in CONFIGURE COINCIDENCE under CONFIGURATION pull down menu if necessary.
5. Place a lid from a 96 well plate onto the stage on position A and select FILL PLATE.
6. Verify sort results by microscope.
7. If results are within specifications, press the Load Plate button and place the new collection device into position. Sample is ready to be dispensed, see section 4.6.5 OPERATION.
8. Refer to section 7.13 SORT PARAMETERS, if results are not within specifications.

4.6.5 OPERATION

1. Select the number of events to be sorted per WELL.
2. Click on the SORT GRID AREA to define the wells to be filled.
3. Click the FILL PLATE button to start sorting.
4. Remove filled plate when finished and replace with a new one.

5. Store data acquisition for each plate to be analyzed in collection mode. Otherwise, continue dispensing in setup mode (data cannot be stored)
6. Continue sorting by clicking the FILL PLATE button.

NOTE: If the CLEAN button is selected during sorting, there will be a back flush into the sample cup from the cleanout bottle that creates a momentary disturbance in the sample flow. In order to avoid contamination, the stage will move to the next well. The sorting to that well may be inaccurate.

CAUTION: Monitor sample volume throughout the sorting. An empty sample cup will cause unwanted spraying to occur onto the system.

CAUTION: If you are dispensing without storing the data, it is advisable to save settings for different samples. When loading a file from the File menu, instrument settings stored with the file will be loaded. This might change Stage Alignment, Gain settings, PMT voltages, markers, and regions of the analysis and sorting.

4.7 ADDING SAME SAMPLE DURING OPERATION

TIP!

If sorting a different sample type than the previous run, the sample cup must be rinsed thoroughly with clean, deionized/distilled water.

TIP!
If sorting a different sample type than the previous run, or sorting criteria has changed, adjust the sorting parameters as necessary in setup mode.

4.7.1 ADDING TO THE SAMPLE CUP

1. Close the sample valve by unchecking the sample valve checkbox (Acquisition is not stopped).
2. Detach pressure tubing from the top of the sample cup and remove cap.
3. Fill the sample cup. Replace the cap and firmly tighten.
4. Re-attach the pressure tubing.
5. Wait 30 seconds for sample cup to repressurize.
6. Restart sample valve by re-checking the checkbox.
7. Continue sorting.

4.8 ADDING SHEATH REAGENT DURING OPERATION

CAUTION: The COPAS *SELECT* is a pressurized system.

1. Close the sample and sheath valves by unchecking the sasmple and the shearh checkboxes. Check the sheath pressure status field beneath the Pressure Status heading to make sure pressure is below 1.0 PSI before opening the sheath container.
2. Open the cap of the sheath container and fill as needed. Replace the cap and firmly tighten.
3. Allow 1-2 minutes for system to repressurize.

4. Open the sheath valve to reestablish sheath flow.
5. Click the CLEAN button to remove any bubbles from the sheath line.
6. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
7. Initiate sorting when pressures reach appropriate levels.

4.9 MANUAL SORTING

Manual sorting is an alternate method for determining sort efficiency or sampling without having to dispense into a multiwell plate.

1. Prepare the instrument and run sample according to previous operating instructions Steps 4.1 through 4.4.
2. Input the appropriate number in /WELL field, telling the instrument to dispense this numbers of objects.
3. Place a standard microscope slide under the sort opening on the waste tray.
4. Click the MANUAL SORT button.
5. View the drop using a microscope to determine if the sorting parameters are optimal.
6. If necessary, change the number of objects in the /WELL field.
7. Place a collection container under the sort opening on the waste tray and click the MANUAL SORT button to continue.

NOTE: A position on a multiwell plate area grid can be used to define a certain position to sort on a Petri dish or other collection container that can be placed on the stage.

4.10 CLEANING

If flow rate decreases or any other flow problems occur, click the CLEAN button to clear the sample valve prior to attempting any troubleshooting of the instrument. See section 13 TROUBLESHOOTING for other options.

Whenever biological sample has been run through instrument it is necessary to perform system cleanse with any one or combination of cleansing reagent protocols: bleach procedure, ethanol sterilization, or cleaning solution, followed by thorough rinsing with deionized/distilled water. See section 6 for detailed cleaning protocols.

If biologicals were not run during instrument use, follow Daily Maintenance Procedure (section 6.1) to cleanse sample cup and flow cell prior to system shutdown.

TIP!
If system is being cleaned in preparation for shut-down, the operator may want to turn off the laser power and allow the cooling fan to start cooling the laser. This can be done from the software, under the Tools dropdown menu, selecting the Run External Laser item, and clicking the STOP button. This turns off the power to the laser but leaves the cooling fan working.

4.11 INSTRUMENT SHUT DOWN

1. After system has been properly cleaned, see section 6 Maintenance, click the STOP button twice. This stops the software.
2. Close the COPAS software program.
3. Purge system pop-up window appears (figure 4.2). Click the SHUTDOWN WITHOUT PURGING button.

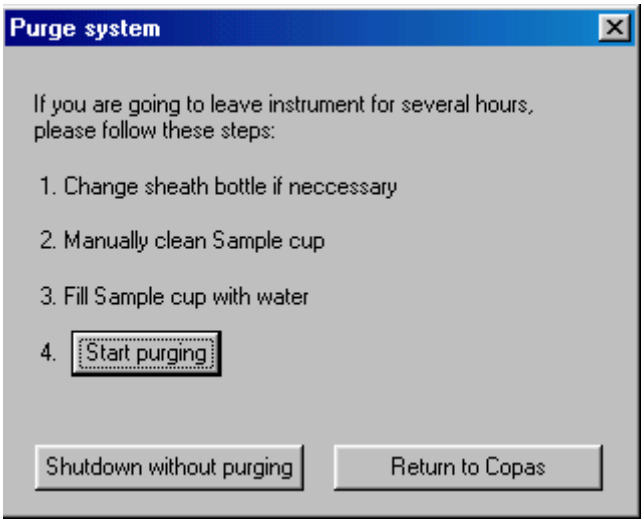


Figure 4.2: Screen capture of Purge system window.

4. Turn the instrument OFF by using the main power switch.
5. Clean external surfaces of any spillage using a water-dampened lint free cloth.
6. Switch the external laser unit(s) OFF by pushing the power button(s) to the OFF position. If present, the argon/ion laser should be turned off by pressing the RED button after cooling has finished.
7. Shut down the computer and monitor.
8. Switch OFF the air-compressor.

5 AVAILABLE OPTIONS

5.1 PROFILER II

For Profiler II operation refer to our specific Profiler II Manual.

5.2 ReFLx SAMPLER OPTION (Recommended for the COPAS *B*IOSORT)

5.3 SAMPLE RECOVERY MODULE

Sampled objects that were not dispensed during instrument operation are normally transferred to a waste collection bottle (or directly into a sink or drain).
The Sample Recovery Module utilizes a cell strainer (40, 70, or 100 micron) to filter the non dispensed objects while allowing the liquids to flow to waste.
Objects retained in Sample Recovery Module can be used for:

- Negative control
- Re-dispensed for optimal recovery
- Sorting at different life stages

6 MAINTENANCE

The following contains the Daily Maintenance procedure and should be performed by the Operator at least once daily when the instrument is in use. If the COPAS instrument will be idle for one to two weeks, follow a short term maintenance schedule as follows: discard the left over sheath fluid and run the instrument with deionized/distilled water in the sheath container and the sample cup for 10 minutes every two/three days. Prior to operation, perform the Bleach Procedure according to section 0 REPLACING THE WASTE PUMP TUBING.

If the COPAS instrument will be idle for greater than two weeks, it is recommended that the short term maintenance schedule also be followed. Prior to operation, perform the Bleach Procedure according to section 0 REPLACING THE WASTE PUMP TUBING. Daily, short/long term shutdown, and sterilization maintenance worksheets are found in the APPENDIX section of this manual. Union Biometrica recommends that these be used to store important instrument information. Recording performance data regularly can aid in the identification of subtle shifts in readings, which may indicate system problems. Make copies of these pages as needed.

6.1 DAILY MAINTENANCE PROCEDURE

NOTE: Do not leave sample or Cleaning Reagent in the instrument overnight.

1. Close the SAMPLE and SHEATH valves by removing check from these checkboxes.
2. Detach the sample tubing and remove cap from sample cup.
3. Discard any remaining sample and wash the sample cup twice with deionized water.
4. Fill the sample cup with approximately 100 ml of Cleaning Reagent. Replace the cap and pressure tubing and firmly tighten the cap.
5. Re-check the SAMPLE VALVE checkbox. The warning message, “Caution, this Operation will contaminate the Flow Cell if Sample is Present”, will appear. Click OK.
6. Process the Cleaning Reagent until the sample cup is nearly empty.
7. Close the SAMPLE VALVE by removing the check from the checkbox.
8. Open the sample cup, remove remaining Cleaning Reagent and rinse with deionized/distilled water 2 times.
9. Fill sample cup again with about 100 ml of deionized/distilled water and firmly tighten the cap.
10. Attach sample tubing to the sample cup.
11. Re-check the SAMPLE VALVE checkbox. Do not allow the sample cup to run dry.
12. Click the STOP button twice.
13. Clean external surfaces of any spillage using a water-dampened lint free cloth.

CAUTION: Do not remove or clean any of the electrical connectors or cables of the COPAS *SELECT*.

6.2 BLEACH PROCEDURE

1. Close the SAMPLE and SHEATH VALVES by removing the chek from their checkboxes.
2. Detach the air pressure tubing and remove cap from the sample cup.
3. Discard any remaining sample.
4. Fill the sample cup with 20 ml of deionized/distilled water and firmly tighten the cap.

5. Attach air pressure tubing to the sample cap.

6. Re-check the SAMPLE VALVE checkbox. The warning message, 'Caution, this Operation will contaminate the Flow Cell if Sample is Present", will appear. Click OK.

7. Process the water until the sample cup is nearly empty.

8. Close the SAMPLE VALVE by removing the check from its checkbox.

9. Detach the air pressure tubing and remove cap from the sample cup.

10. Fill about half the sample cup with 50% bleach solution (final hypochlorite concentration should be ~2.5%), refer to section 10 REAGENTS, for bleach concentration specifications, replace the cap and firmly tighten.

11. Attach air pressure tubing to the sample cap.

12. Re-check the SAMPLE VALVE checkbox. Click OK when error message appears to process the bleach until the sample cup is nearly empty.

13. Close the SAMPLE VALVE.

14. Open the SHEATH VALVE and allow sheath to process for 3 minutes.

15. Detach the air pressure tubing and remove cap from the sample cup.

16. Rinse the sample cup twice with ~20ml of deionized/distilled water aspirating contents between rinses.

17. Fill the sample cup again with 20 ml of deionized/distilled water and firmly tighten the cap.

18. Attach air pressure tubing to the sample cap.

19. Re-check the SAMPLE VALVE checkbox. Process the water for several minutes without letting the sample cup to run dry.

20. Click STOP once.

21. Clean external surfaces of any spillage using a water dampened lint free cloth.

6.3 COMPLETE STERILIZATION PROCEDURE

Complete sterilization of the instrument involves sterilization of all fluid lines as well as surfaces that the sample may contact. There are four main components of complete sterilization: Bleach sterilization, Ethanol sterilization, Rinsing protocol, and surface sterilization. The user can choose which combination of sterilization techniques to perform as needed or on a daily basis.

Bleach Sterilize the Sheath and Clean/Flush bottles.

1. Close the SAMPLE and SHEATH VALVES by removing checks from their checkboxes.
2. Detach the fluid lines from the Sheath and Clean/Flush containers.
3. If sterility filters are present, detach and remove any sterility filters from the Sheath and Clean/Flush lines.
4. Remove the cap from the Sheath and Clean/Flush containers, and empty their contents.
5. Rinse each container with deionized/distilled water.
6. Fill the Sheath container with 1 liter of freshly made 50% Bleach Solution (final hypochlorite concentration should be ~2.5%).
7. Fill the Clean/Flush container with 200ml of 50% bleach solution.
8. Carefully swirl the bleach in each container to sterilize all the interior surfaces.
9. Reattach the fluid lines to both the Sheath and Clean/Flush containers. Replace and firmly tighten the caps and allow the system to pressurize.
10. Open the SHEATH VALVE by clicking the sheath valve checkbox. Allow a few minutes for the bleach to flood the Sheath line and Flow Cell.

11. Depress the Clean button several times to send bleach through the Clean/Flush lines and up into the sample cup.
12. Monitor the waste output to ensure the bleach solution is flowing through the system.
13. After bleach solution has processed for 10-20 minutes, close the SHEATH VALVE by unchecking the SHEATH VALVE checkbox.

Bleach Sterilize the sample cup as follows:

14. Click OFF the SAMPLE and SHEATH VALVE checkboxes.
15. Detach the air tubing and remove cap from the sample cup.
16. Discard any remaining sample.
17. Rinse the cup with 40 ml of deionized/distilled water.
18. Fill the cup with 50% Bleach Solution (final hypochlorite concentration ~2.5%).
19. Reattach the air tubing to the sample cup.
20. Re-check the SAMPLE VALVE checkbox. The warning message, "Caution, this Operation will contaminate the Flow Cell if Sample is Present", will appear. Click OK.
21. Process the Bleach solution until the sample cup is nearly empty.

Rinse all containers with clean water.

22. Press the stop button once to stop all fluids from moving through the system.
23. Rinse the Sheath and Clean/Flush containers with copious amounts of clean water. Then fill the Sheath container with 1L of clean water, fill Clean/Flush container with 500ml of clean water.
24. Replace caps to the Sheath and Clean/Flush containers and allow a minute for the system to repressurize.
25. Open the Sheath valve using the manual control checkbox and allow the water to rinse the Sheath lines.
26. Press the CLEAN button several times to allow water to rinse the Clean/Flush tubing.
27. Rinse the Sample cup with copious amounts of clean water.
28. Fill the Sample cup with 40ml of deionized/distilled water. Replace the cap and attach the air pressure tubing.
29. Close the SHEATH VALVE then open the SAMPLE VALVE using the manual control checkbox. Allow deionized/distilled water to rinse the Sample tubing until Sample cup is nearly empty.
30. Close the SAMPLE VALVE and open the SHEATH VALVE and allow water to rinse the system for 20-30 minutes. Do not allow sheath bottle to completely empty.
31. Close the SHEATH VALVE by removing the checkmark from the check box.

Ethanol Sterilize the Sheath and Clean/Flush containers.

32. Ensure SAMPLE and SHEATH VALVES are closed (check marks are removed from checkboxes).
33. Detach the fluid lines from the Sheath and Clean/Flush containers.
34. If sterility filters are present, detach and remove any sterility filters from the Sheath and Clean/Flush lines.
35. Remove the cap from the Sheath and Clean/Flush containers, and empty their contents.
36. If necessary, rinse each container with deionized/distilled water.
37. Fill the Sheath container with 1 liter of 70% Ethanol.
38. Fill the Clean/Flush container with at least 200ml of 70% Ethanol.
39. Carefully swirl the Ethanol in each container to sterilize all the interior surfaces.
40. Reattach the fluid lines to both the Sheath and Clean/Flush containers. Replace the container caps and allow the system to pressurize.

41. Open the SHEATH VALVE by clicking the sheath valve checkbox. Allow a few minutes for the Ethanol to flood the Sheath line and Flow Cell.
42. Depress the Clean button several times to send Ethanol through the Clean/Flush lines and up into the sample cup.
43. Monitor the waste output to ensure the Ethanol is flowing through the system.
44. After Ethanol has processed for 10-20 minutes, close the SHEATH VALVE by removing the check from the SHEATH VALVE checkbox.

Ethanol Sterilize the Sample cup.

45. Close the SAMPLE and SHEATH VALVES by removing the checks from their checkboxes.
46. Detach the air tubing and remove the cap from the sample cup.
47. Discard any contents from the sample cup.
48. If necessary, rinse the cup with 40 ml of deionized/distilled water several times.
49. Fill the cup with 70% Ethanol to the very top of the cup.
50. Aspirate the ethanol to just below the threads of the cap.
51. Spray the cap with ethanol before replacing the cap.
52. Reattach the air tubing to the sample cup.
53. Re-check the SAMPLE VALVE checkbox. The warning message, "Caution, this Operation will contaminate the Flow Cell if Sample is Present", will appear. Click OK.
54. Process the Ethanol until the sample cup is nearly empty, removing the sample check to close the SAMPLE VALVE.

Apply sterility Filters to the SHEATH and CLEAN/FLUSH lines using sterile technique.

55. Press the stop button once to stop all fluids from moving through the system.
56. Sterilize the area by spraying all the surfaces around the Sheath and Clean containers with 70% ethanol.
57. Wearing Ethanol sprayed gloves, open and remove the Sheath sterility filter from its bag and locate the downstream end of the filter (an arrow on the sterility capsule points to the down stream fluid fitting).
58. Carefully remove the foil at the downstream end of the sterility filter taking care not to touch it to any surfaces.
59. Disconnect the sheath line fitting from the sheath bottle taking care not to touch it to any surfaces.
60. Spray both ends liberally with ethanol before fitting them together.
61. Leave the upstream end of the sterility filter wrapped in foil.
62. Repeat these steps to apply the sterility filter to the Clean/Flush container.
63. Remove the caps to the Sheath and Clean/Flush containers. Remove any remaining ethanol from the Sheath and Clean/Flush containers before rinsing each with sterile deionized/distilled water.
64. Carefully remove the foil of the Sheath line sterility filter. Spray both connectors (on sterility filter and sheath container) before attaching the container to the sterility filter.
65. Repeat this procedure for the Clean/Flush sterility filter and container.

Rinse all lines with Sterile deionized/distilled water.

66. If not already performed, remove caps and fluid line connections from the Sheath and Clean/Flush containers and rinse with Sterile deionized/distilled water.
67. Refill the Sheath container with 1 liter of sterile deionized/distilled water.
68. Refill the Clean/Flush container with at least 200ml of sterile deionized/distilled water.
69. Spray each cap (inside and out) with 70% ethanol before replacing the cap to the appropriate container and allow a minute for the system to repressurize.

70. Open the SHEATH VALVE, allowing the water to rinse the fluid lines for several minutes. Observe the waste pump tubing to ensure fluids are moving through the system, increase the SHEATH PRESSURE if necessary to establish sheath fluid movement.
71. If necessary debubble the sheath sterility filter and fluid line: Hold the downstream end of the sterility filter upwards so that any bubbles contained in the filter will exit the downstream end. Alternatively, using sterile technique, the bleed cap on the filter capsule may be momentarily loosened to allow air bubbles to escape. Make sure to firmly tighten the 'bleed cap' when done.
72. Turn off the SHEATH VALVE. Make a crimp in the sheath line just downstream of where the sterility filter is attached suing a tube clamp or strong paper fastener to hold the crimp in place.
73. Press the CLEAN BUTTON several times to allow the sterile water to wet the filter and rinse the clean line.
74. Debubble the clean line and sterility filter as described in step 68 (for the sheath line) by pressing the clean button several times to induce the water to rinse through the clean lines.

Note: Sterility filters require about 24 hours to become sufficiently wet. It is good practice to debubble the sheath and clean lines 24hours after sterility filters are newly attached to the instrument.

75. Remove the clamp on the SHEATH line and open the SHEATH VALVE to allow the water to continue to rinse the sheath lines for 10-30 minutes. Clean button may be depressed several more times during this time period but do not allow the containers to fully empty.

Rinse the Sample cup with sterile water.

76. Click OFF the SAMPLE and SHEATH VALVE checkboxes.
77. Detach the air tubing and remove the cap from the sample cup.
78. Discard the ethanol from the sample cup.
79. Rinse the cup with sterile deionized/distilled water several times.
80. Fill the cup with sterile water and replace the cap.
81. Reattach the air tubing to the sample cup.
82. Re-check the SAMPLE VALVE checkbox. The warning message, "Caution, this Operation will contaminate the Flow Cell if Sample is Present", will appear. Click OK.
83. Process the water until the sample cup is nearly empty.
84. Click the STOP button once to stop all fluids moving through the system.

Establish appropriate Sheath flow.

85. While no fluids are moving through the system, carefully open and empty the sheath and clean/flush containers of the sterile water.
86. Refill each container with the appropriate sheath fluid and replace the container caps.
87. Open the SHEATH VALVE and allow sheath to flood the tubing and allow it to process for several minutes before closing the SHEATH VALVE.
88. With the sheath line crimped (see step 71), press the clean button several times to allow sheath to process through clean filter and tubing.
89. Remove the crimp on sheath line, open SHEATH VALVE and establish adequate sheath flow. Take a 1 minute collection of sheath fluid processed through the system, adjusting the tension on the sheath regulator to establish sheath flow to give a 9-10ml/minute collection of sheath fluid.
90. System is ready to run samples or control particles. However if a sterile sort is preferred, waste tray and lines should be sterilized.

Sterilize Waste tray and sample recovery cup with ethanol.

91. Ensure no fluids are moving through the system. Carefully remove the waste tubing from the base of the waste tray.
92. Carefully slide the waste tray from its holder to completely remove it from the instrument.

93. Using 70% ethanol dampened KIM wipes and/or Q-tips, sterilize all the surfaces of the waste tray holder: sliders, divertor, and the aperture below the flow cell nozzle.
94. Thoroughly spray the waste tray with 70% ethanol to sterilize all its surfaces before putting it back in position.
95. Fill a conical tube with 70% ethanol. Dip the waste tubing into the conical tube and allow the waste pump to draw all of the ethanol through the tubing, sterilizing all the internal surfaces.
96. Allow the tubing to air dry or rinse it in a similar manner with sterile water.
97. Ethanol cleanse the tubing end before refitting it to the waste tray.
98. Open and empty the sample recovery cup and remove the cell strainer from its housing.
99. Refill the sample recovery cup to its brim with 70% ethanol and screw it back in place. The waste pump will draw the ethanol through the exit tubing, sterilizing its inner surfaces. Notice not all the ethanol will be removed from the cup.
100. Remove and empty the recovery cup and rinse with sterile water.
101. Fit a new cell strainer in place and replace the sample recovery cup into its position. Waste pump will draw sterile water through the tubing to rinse this part of the instrument.
102. Ensure the entire waste line is reconnected properly. Open the Sheath Valve and watch that the waste stream moving through tubing to recovery cup and to the waste collection bottle.
103. At this point the instrument and waste areas have been sterilized and the system is ready to run and dispense samples.

CAUTION: Do not remove or clean any of the electrical connectors or cables of the COPAS instrument.

REPLACING THE WASTE PUMP TUBING

This procedure is periodic, depending on usage. Visual inspection of the waste pump tubing is recommended on a regular basis. The appearance of dry, cracked tubing or pooling under the waste tray is an indication that the waste pump tubing needs to be replaced. Follow the steps below to change the tubing.

1. Click the STOP button twice in the COPAS software if the instrument is ON.
2. Turn off the main power of the COPAS system.
3. Remove waste pump tubing. First remove the tubing from the bottom of the waste pump housing. Next release tubing from the top of waste pump, taking care not to put stress on the T-connector here.
4. Using the old tubing as a guide, cut a new length of tubing and attach to the connectors.
5. Install the new tubing by inserting first the top then the bottom connectors into their respective positions around the waste pump.
6. Turn on the main power of the COPAS system.
7. Open the sheath valve to verify that fluid is pumping through the waste pump tubing to the waste bottle and, if not, shorten the new tubing.

7 COPAS SOFTWARE

This manual has been developed for COPAS Software Revision 5.26.
Previous software revisions may have subtle differences in appearance, drop down menu options, and names of control features. If you are unsure of which software revision is currently installed on the COPAS PC, this information may be accessed using the ABOUT drop down menu.

7.1 START UP

The following picture shows the main screen of the COPAS software. The General, Acquisition, and Sort Controls discussed in the following sections are accessed using buttons and checkboxes located in the right hand column of the main software screen.

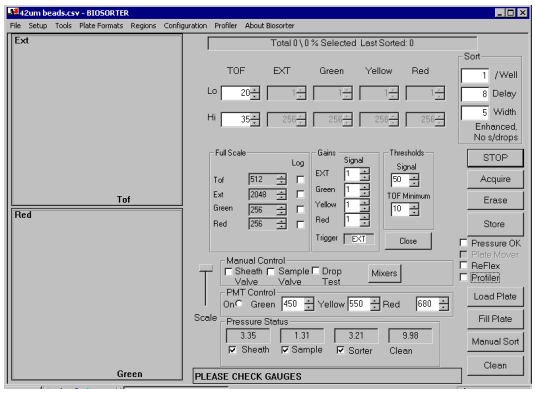


Figure 7.1: Screen capture of the COPAS Software.

7.2 GENERAL CONTROLS

7.2.1 START/STOP

Click the START/STOP button located in the upper right hand corner of the screen once using the computer mouse to turn ON the sheath, sample, and sorter pressures. Subsequent clicks to this button STOP any automated operation in progress. Two consecutive clicks turn the pressures OFF.

7.2.2 PRESSURE OK

Click the PRESSURE OK checkbox, also located in the upper right hand corner of the main screen, when pressures equilibrate to desired levels. Pressures can be viewed and controlled in the Pressure Status field. Once PRESSURE OK is selected the system will automatically begin priming the flow cell.

7.3 ACQUISITION CONTROLS

7.3.1 ACQUIRE/PAUSE

Click the ACQUIRE button to start the sheath and sample flows. Once the ACQUIRE button has been pressed, the title changes to PAUSE. The user can click the PAUSE button to stop acquiring data while maintaining sheath and sample flow. Sample and sheath fluid will continue to flow through the flow cell.

7.3.2 ERASE

Click the ERASE button to clear acquired data and to restart the event counter.

7.3.3 STORE

Click the STORE button to store a data file (stored in lmd = List Mode Data format, stored in txt = Text Format, stored as a BSRT file for reanalysis in the COPAS software) containing the number of events as indicated by the storage mode

selected. The default SAVE TO file folder is the program directory. It is highly recommended to redirect the program to store data files in a new data directory. Refer to Section 8 for discussion of DATA STORAGE.

7.4 SORT CONTROLS

7.4.1 PLATE MOVER

Click the PLATE MOVER checkbox to activate the (optional) Plate Handler.

7.4.2 LOAD PLATE

Click the LOAD PLATE button to move the stage out to the front left to accept a new plate for dispensing.

7.4.3 FILL PLATE

Click the FILL PLATE button to begin sorting to a multiwell plate as defined in section 7.13.5 SORT GRID AREA. The stage will self-calibrate by returning briefly to the home position (fully left and front) and then move to the first well to be filled.

7.4.4 MANUAL SORT

Click the MANUAL SORT button for continuous bulk sorting of 1 to 50,000 objects. MANUAL SORT is used for sorting the specified number of events as defined in the per WELL field located under the Sort Parameters heading. See section 4.9 MANUAL SORTING for operation.

7.4.5 CLEAN

Click the CLEAN button to initiate a cleaning sequence. The cleaning sequence forces a nominal amount of sheath back through the flow cell into the sample cup. If CLEAN is initiated while sorting into a multiwell plate, the COPAS will skip sorting at the current well in order to avoid data contamination.

7.5 MANUAL CONTROLS

7.5.1 SHEATH AND SAMPLE VALVE

Click the ACQUIRE button to control the SHEATH and SAMPLE VALVES automatically. If manual control is desired (e.g. during cleaning), both the SHEATH VALVE and SAMPLE VALVE can be controlled manually by checking and un-checking the valve control checkboxes located in the PRESSURE STATUS FIELD. A check mark in the field indicates the valve is open.

7.5.2 MIXERS

Click the MIXERS checkbox located in the lower left hand quadrant of the main screen to start/stop the stir bars in the sample cup. While the stir bars are stopped, the mixing speed can be adjusted. The values indicate the percentage (40%, 60%, 80% or 100%) of the maximum stir bar speed specified in the program's initialization file.

7.5.3 DROP TEST

Click the DROP TEST checkbox to verify proper drop formation and placement before sorting begins. The number of droplets specified in the per WELL field will be deposited. This function is also available in the ALIGN PLATE HANDLER & STAGE option located under the TOOLS drop down menu.

7.6 PARAMETERS FOR ANALYSIS

When an object passes through the laser beam, signals are generated and collected. A photodiode collects the 670-laser beam emission (or the optional 635 nm laser), which is represented by two parameters: TOF and EXT. These parameters are described below. Photon Multiplier Tubes (PMTs) collect fluorescence emission generated from excitation of fluorochromes by the 488 or 514-laser beam. The output signals are converted into a 256-channel resolution represented on each individual histogram.

TIP!
Due to the wide range in size of particles and multi-cellular organisms, it may be necessary for the user to adjust the signals and amplification. Refer to sections 7.7 ADJUSTING GAIN VALUES, 7.11 SORT CRITERIA DISPLAY and 7.13 SORT PARAMETERS.

7.6.1 TOF (TIME OF FLIGHT)

Measures the amount of time the instrument microprocessor is detained in the analysis of a signal. TOF is usually an indicator of the length of an object.

7.6.2 EXT (EXTINCTION)

Measures the decrease in laser light when a particle or object passes through the laser beam. Extinction is an indicator of the size and internal structure of the object.

7.6.3 GREEN

Specifies a (changeable) filter of 510 nm optimum with a band width of 23 nm. Emission can be collected from various fluorochromes.

7.6.4 YELLOW

Specifies a (changeable) filter of 545 nm optimum with a band width of 25 nm. Emission can be collected from various fluorochromes.

7.6.5 RED

Specifies a (changeable) filter of 610 nm optimum with a band width of 20 nm. Emission can be collected from various fluorochromes.

7.7 ADJUSTING GAIN VALUES

Select ADJUST GAIN VALUES from the TOOLS drop down menu. A new main screen view will replace the multiwell plate image.

7.7.1 SCALE

Displays the selected data. All parameters' scales are adjustable from 256 channels (full scale) up to as much as 65536 channels. You can also choose to display data using a log scale of 3, 4, or 5 decades to view data. Scale settings are embedded in lmd file settings. Adjusting the scale does not change the stored raw data in text file.

7.7.2 GAINS

The GAINS setting changes the amplification of the original signal. A GAIN setting of 1 represents an amplification of 1, setting of 2 doubles the signal, 3 triples and so on up to 5 times amplified. When attenuating signals, the trigger Threshold may need to be adjusted as well.

7.7.3 THRESHOLD

Sets the position of the 0 channel (of the 256) and affects the sensitivity of the system. The Threshold is set on the Trigger parameter selected (by default, the trigger is set on the EXT signal). The Threshold can be changed from the EXT signal to one of the active fluorescence parameters using the TOOLS drop down menu. Refer to section 7.14 ADDITIONAL MENU OPTIONS.

7.7.4 MINIMUM

Applies to the TOF parameter and prevents processing of very small signals. This setting is the minimum required signal (from trigger source) that each object must exceed in order to be considered an object. The TOF minimum can be adjusted related to the size of the objects analyzed (1-8000 units). It can be used to eliminate analysis of debris that may impede sorting. See 7.11 SORT CRITERIA DISPLAY for more information.

TIP!
Small objects or debris give rise to signals with smaller TOF values. Using an appropriate THRESHOLD for a given GAIN setting will ignore these small objects.

NOTE: Return to the plate display by clicking the CLOSE button when finished setting parameters. Instrument settings can be stored by saving the file using the FILE drop down menu or automatically when data is stored.

7.8 PMT CONTROL

Since fluorescence emission is a relatively weak signal, PMT Control applies additional voltage for signal amplification to the PMTs (compared to TOF and EXT). The factory configuration for the PMT filter assembly assigns a GREEN filter to PMT 1, a YELLOW filter to PMT 2, and a RED filter to PMT 3. The voltage settings are adjustable from 300 to 1100 (a setting of 0 turns the PMT off). A non-fluorescent control sample can be used to determine a baseline PMT setting.

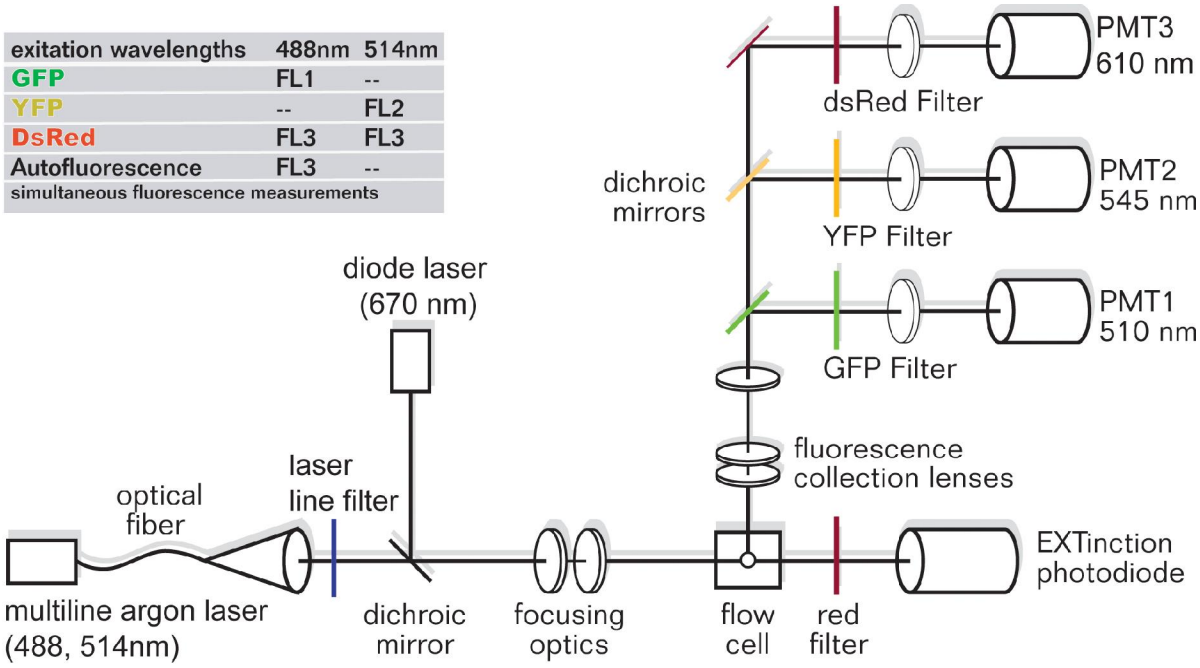
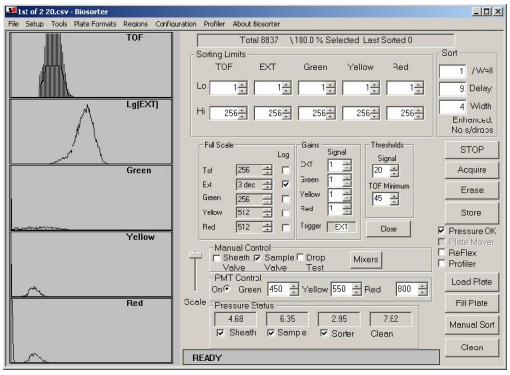


Figure 7.2: Top View of Optics Layout displaying optics setup with 670 laser and mulitline 488/514nm laser. Specific laser and PMT filter combinations may vary based on which instrument options were purchased for your system.

7.9 DATA REPRESENTATION

Displays data in two possible ways: a single parameter representation, called HISTOGRAM, or a dual parameter representation, called DOT PLOT.



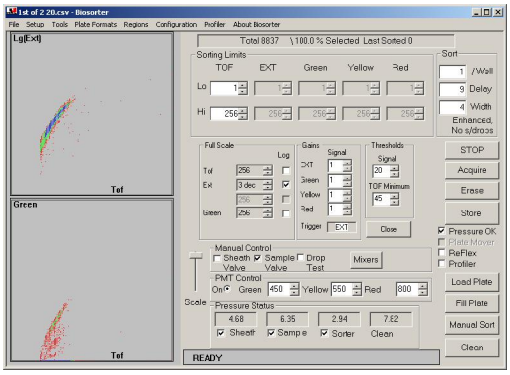


Figure 7.3: Screen captures of COPAS software main screen (HISTOGRAM view Left, DOT PLOT view Right)

NOTE: Double click in the center of the data plots to change the display from five single parameter histograms to two, dual parameter dot plots, or vice versa.

7.10 SCALE BAR

SCALE adjustments can be made to change the vertical scale of the histogram displays if events go off-scale. In dot plot mode it will decrease the density of plotted events. Scaling only changes the software display of raw data. The scaling does not affect the number of events identified on the counter or stored in the data file.

7.11 SORT CRITERIA DISPLAY

7.11.1 HISTOGRAMS

1. Select the GATING REGION by adjusting the lower and upper markers of the TOF parameters between 1 and 256 in order to eliminate analysis of specific events (such as debris). Click on left and/or right markers dragging them to the appropriate position to create upper and lower boundaries around data events you wish to display. Note: Only data events within these boundaries will be displayed on lower histograms and on the dot plots.
2. Set the SORT CRITERIA by adjusting the lower and upper markers of all parameters between 1 and 256 in order to obtain the desired population. Again dragging left and right markers will allow you to include/exclude objects of interest to dispense.

TIP!
The TOF parameter is always active and can be used for gating, either alone or in combination with the dot plots. Gating using TOF eliminates those events that fall outside of the region from being displayed on the subsequent histograms and/or dot plots.

7.11.2 DOT PLOTS

A subset of data can be defined through creating a "gate" which is a numerical or graphical boundary that can be used to define the characteristics of objects to include for further analysis. See section 7.14.5 REGIONS for more information.

1. To create a gate, make sure appropriate data parameters are displayed on the dot plots. If necessary, change the parameters to display using those available in the CONFIGURATION drop down menu. Select the Gating Region by selecting DEFINE GATE REGION from the REGIONS drop down menu. The cursor will change to a "+" and it will then be possible to drag and click the mouse within the first (upper) dot plot on the main screen to form a polygonal region around the desired sub-population for analysis.
2. Change an existing Gating Region by selecting EDIT GATING REGION or RESET TO ALL from the REGIONS drop down menu.
3. Select the Sorting Region by selecting DEFINE SORT REGION from the REGIONS drop down menu. The cursor will change to a "+" and it will be possible to drag and click the mouse within the second (lower) dot plot on the main screen to form a polygonal region around the desired sub-population for sorting.
4. Change an existing Sort Region by selecting EDIT SORT REGION or RESET TO ALL from the REGIONS drop down menu.

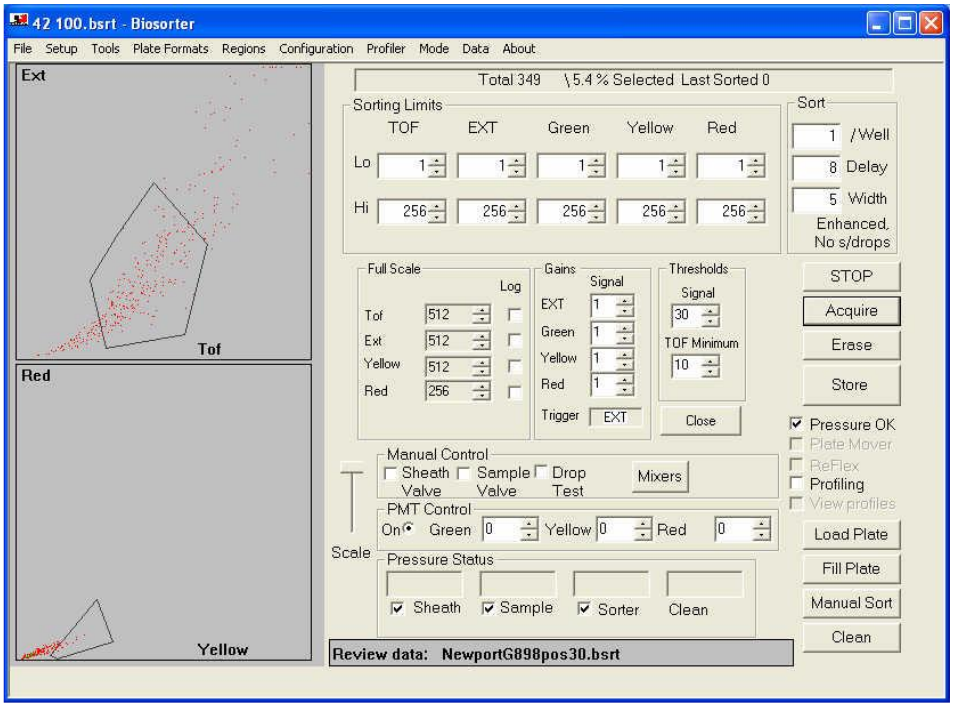


Figure 7.4: Screen capture of COPAS software main screen DOT PLOT view with Gate and Sort Regions selected.

TIP!
Any dual parameter combination can be selected on the first (upper or Gating) dot plot from the gating parameters in the CONFIGURATION drop down menu. Gating eliminates those events that fall outside of the region from being displayed on the subsequent histograms and/or Sorting dot plot.

TIP!
Any dual parameter combination can be selected on the second (lower or Sorting) dot plot from the sorting parameters in the CONFIGURATION drop down menu. The sorting window indentifies the objects selected for dispensing.

7.12 TOTAL 0 \ 0% SELECTED

TOTAL, located in the upper center of the screen, displays the total number of objects detected by the instrument during acquisition.

SELECTED displays the percentage of objects that fulfill all sort criteria (within Gate and Sort Regions) from the total events detected.

7.13 SORT PARAMETERS

7.13.1 PER WELL

Indicates the number of objects to be dispensed per well if sorting into a multiwell plate, or the total number of objects to be dispensed during manual sorting into a bulk receptacle. The acceptable range is 0 to 50,000.

7.13.2 SORT DELAY

Indicates the amount of time, in milliseconds, from analysis of the object to the sort command. The value for SORT DELAY is COPAS platform and sample dependent. By performing a test dispense, the delay can be checked (See section 4 OPERATING THE COPAS).

7.13.3 SORT WIDTH

Determines the volume of fluid the selected object is contained in based on the time, in milliseconds, that the SORT VALVE is off. Sort width can also be described as drop volume.

7.13.4 COINCIDENCE METHOD AND SUPERDROPS

Ensures that there are no additional objects in sample stream that may affect accurate sorting. Coincidence occurs when two or more objects pass the laser beam so close to each other that both objects could be contained within the same dispensed drop of liquid and fall into the same well. See section 7.14 ADDITIONAL MENU OPTIONS.

7.13.5 SORT GRID AREA

Displays a multiwell plate template on the main software screen. Clicking in the well fields defines the wells to be filled and the fill sequence, for example when well A1 is clicked, a number will appear indicating this well will be filled. This number designates the filling order. Any well left blank will not be filled. For rapid sequence selection, hold the shift key down and move the mouse over the desired well fields in the desired fill order.

CAUTION: The Sort Width and Coincidence Check time have an immediate effect on the sort yield (effective sort recovery). Minimizing the Sort Width value improves the yield by reducing coincidence. If, however, the Sort Width value is set too low, sortable events may not be contained within the dispensed drop.

7.14 ADDITIONAL MENU OPTIONS

Your COPAS instrument has been customized based upon the options you have purchased. Multiple options are available for the user, which may be accessed via the main screen task bar. The user can select highlighted options on the drop down menu, those options that were not purchased are "ghosted".

7.14.1 FILE MENU

The FILE drop down menu is similar to any Windows format. The following options are located under the FILE drop down menu:

NEW
Allows the user to create a new template and set different instrument settings.

OPEN
Allows the user to open a saved csv file containing previously stored settings.

SAVE & SAVE AS
Allows the user to save the current template and instrument settings as a csv file.

PRINT
Allows the user to print the current screen.

SAVE SCREEN IMAGE
Allows the user to store a "print screen" capture of the software screen. Upon clicking this feature, a "Save as" dialogue box is opened prompting the user to direct a file name and location for storing COPAS software screen capture. The image is saved as a bmp file.

SAVE SERVICE DATA
Should the system identify an error during use, it will automatically prompt the user to SAVE SERVICE DATA. Sometimes the user will encounter an error that the system has not identified. In this case the user can choose the SAVE SERVICE DATA from the File menu to log the system error. Once initiated the user will choose a file name to store the error file and should notify Union Biometrica to diagnose the error.

WRITE NOTES
When selected, WRITE NOTES opens a small dialogue box that allows the user the type in text relevant to the current stored data. The notes are stored in the csv file as well as the txt file. Notes are displayed at the end of the file along with the summary of the instrument settings used during data acquisition.

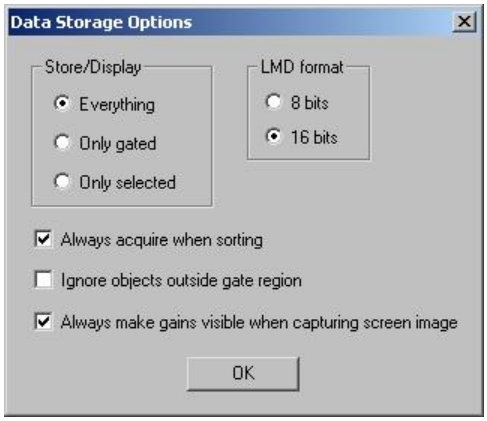
7.14.2 SETUP MENU

The following options are located under the SETUP drop down menu:

WARNING MESSAGES
When a check mark appears next to this feature all warning messages will be displayed as they are encountered during instrument use.

DEFINE PMT LABELS
Allows the user to rename the PMT filters to reflect sample specific or PMT specific nomenclature.

DATA STORAGE OPTIONS
Allows the user to choose how data is displayed and stored.



ALWAYS ACQUIRE WHEN SORTING
When selected, system will always begin data acquisition for data storage once a sort command, either manually or to a plate, has been initiated.

IGNORE OBJECTS OUTSIDE GATE REGION.
When selected, system will not count, display, or store events that do not fall within the gate dot plot polygon. Total Acquired will reflect only events that fall within the drawn gate polygon. % selected will be calculated as percentage of selected from total within gate polygon region.

NOTE: Because system does not determine coincidence of objects it was instructed to "ignore", it may be possible to dispense "ignored" objects in a drop containing the intended sort object.

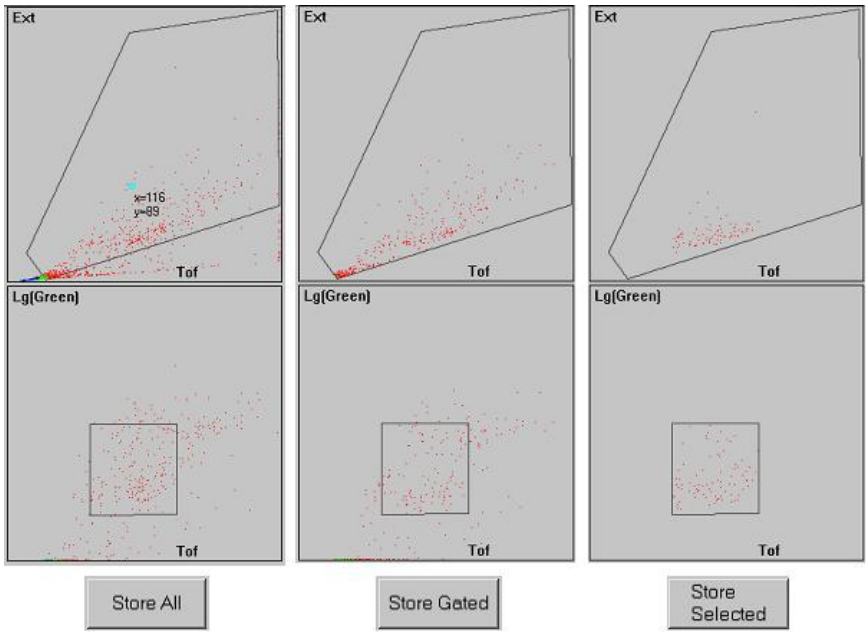
ALWAYS MAKE GAINS VISIBLE WHEN CAPTURING SCREEN IMAGES
When this option is selected a screen image capture will always display the gain settings rather than the sort plate template that may have been visible on the screen.

LMD FILE DATA STORAGE
Select 8 or 16 bit data storage for lmd file.

STORE/DISPLAY

EVERYTHING
All events fulfilling trigger threshold and minimum TOF requirements will be displayed in gate dot plot and stored in txt and lmd files. Once this display storage option is selected, the store button will read STORE ALL.

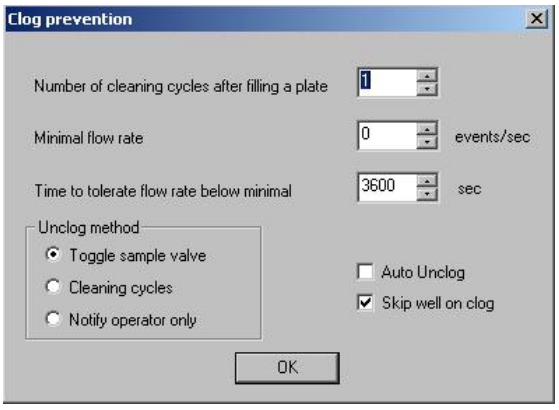
ONLY GATED
Only events that fall within the gate polygon in the gating (upper dot plot) will be displayed in dot plots and stored in txt and lmd files. If this mode is selected, the store button will read STORE GATED.



ONLY SELECTED
Only selected events that fall within both the gate and sort region polygons will be displayed in dot plots and stored in txt and lmd files. Once chosen, the store button will change to read STORE SELECTED.

NOTE: 'Total events Acquired' and '% selected' will reflect all events that fulfill trigger and threshold requirements regardless of whether they fall within gate or sort regions unless user chooses IGNORE OBJECTS OUTSIDE GATE REGION option.

CLOG PREVENTION
Allows the user to activate an auto unclog feature during sorting either manually or to a multiwell plate. When activated, the system will monitor the flow rate to identify when a clog has occurred. Once detected, the system will initiate the selected method for clog removal.



To use:
1. Set the number of clean cycles to run after sorting to a plate. Like pressing the CLEAN button, the system sends a flush of fluid from the clean bottle through the flow cell into the sample cup. User may can choose 1-100 cycles that will automatically run after sorting is completed.
2. Set the minimal flow rate before 'auto unclog' will be initiated. This value should be set quite low in order to accurately identify a clog and initiate auto unclog.
3. Set the time to tolerate flow rate below minimal flow rate. Determine an amount of time with flow rate less than the minimal flow rate before auto unclog is initiated. Limits can be set between 1 second to 1 hour (1-3600 seconds).
4. Choose an unclog method. User can choose to have system either toggle the sample valve, turning it off and on a few times, or initiate a clean cycle (a backflush of fluid through the flow cell into sample cup).
5. Choose whether to have the system skip a well during sort mode if auto unclog is used.
6. Initiate auto unclog by checking the Auto Unclog box.
7. Click OK to implement clog prevention.

NOTE: Consider that flow rate (events/second displayed at bottom of software screen during acquisition) is dependent on identifying objects within the sample stream. If the user has chosen to ignore objects outside of gate region or very restrictive trigger and TOF min requirements, the flow rate may be very low-auto unclog may be triggered.

MIXER'S TYPE

When available, user can choose which mixer motors to run. If two are available, user can select one (DC motor: small, Stepper motor: large) or both.

7.14.3 TOOLS MENU

The following options are located under the TOOLS drop down menu:

ALIGN PLATE HANDLER & STAGE

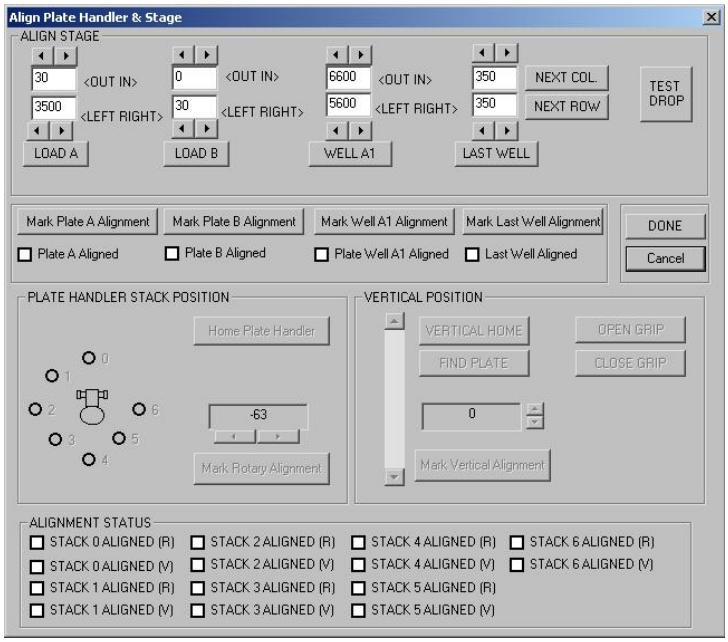
NOTE: The Plate Handler adjustments are only activated when the Plate Handler option is installed and the PLATE MOVER checkbox is activated.

PLATE HANDLER ALIGNMENT

Aligns the Plate Mover, if this option is installed and activated.

STAGE ALIGNMENT

Aligns the stage using numbers, which indicate the position. This is done by typing or clicking the arrows to increase or decrease the position numbers. Clicking NEXT COL or NEXT ROW buttons allows additional positions to be checked. Choose DONE when the stage is aligned, settings will be saved with the document. See section 4.6.3 ALIGN STAGE for instructions for aligning the stage.



LOAD A

Moves the stage to the position to accept a new microtiter plate from the user or the Plate Mover.

LOAD B

Moves the stage to the position to accept a new second microtiter plate from the user or the Plate Mover.

WELL A1

Moves the plate A to the first well collection position.

LAST WELL

Moves the plate A to the last well collection position.

TEST DROP

Dispenses drops onto the designated position (Well A1, Last Well, etc) during Stage Alignment (the number of drops is equal to the number indicated in sort /well window on the main screen.

CAUTION: If the application window is closed by clicking the X button in the upper right corner or by pressing the cancel button, settings will NOT be stored.

RUN CONTROL PARTICLES

Refer to section 4.2 RUNNING CONTROL PARTICLES.

ADJUST GAIN VALUES

Refer to Section 7.7 ADJUSTING GAIN VALUES.

RUN EXTERNAL LASER

Allows control (Run, Standby or Stop) of the primary external laser.

SET TRIGGER CHANNEL

Allows the trigger parameter used for the THRESHOLD to be changed between the EXT signal and one of the active fluorescence parameters.

MAINTENANCE

PRIME REFLX

Sends a flush of liquid through ReFlx Sampler system. Feature will be ghosted if this option was not installed

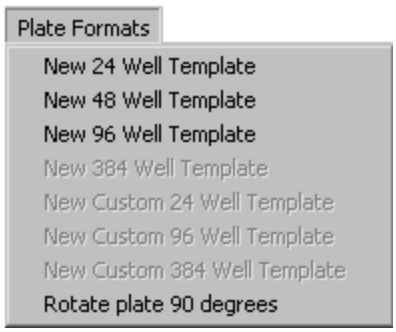
PRIME FLOW CELL

Primes the flow cell when activated with Sheath fluid in order to remove any possible air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

PURGE

Prompts the user (when activated) to clean the instrument prior to instrument shutdown.

7.14.4 PLATE FORMATS MENU



Allows the selection of plate sizes including 24, 48, and 96 wells. Provides the user with the option to obtain a blank SORT GRID without clicking in the well fields. Allows the user to create new custom SORT GRIDS if the SFLEX Software Option has been installed (Custom Templates).

7.14.5 REGIONS

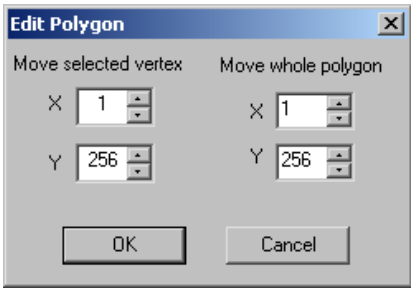
Allows the user to manually draw a polygon for gate and sort regions.

DEFINE A GATE and/or SORT REGION

Using the mouse, draw a multisided polygon around a population of interest by left clicking to define the location of a vertex. After each vertex is added, a line will be drawn connecting the previous vertex to the new one. User must close the polygon by returning to original coordinate for system to accept the region.

EDIT THE GATE/SORT REGION

To modify an existing GATE/SORT region click on EDIT GATE/SORT REGION. The vertices of the polygon will be identified with a small square and a dialogue box will appear displaying the coordinates of the active vertex. Using the mouse, click on a vertex to drag it to a new location or change the coordinates of the vertex by typing in a new coordinate set. Repeat this for each vertex as necessary. The Polygon shape will change while user is modifying the coordinates. Click OK to implement the changes.



RESET GATE/SORT TO ALL

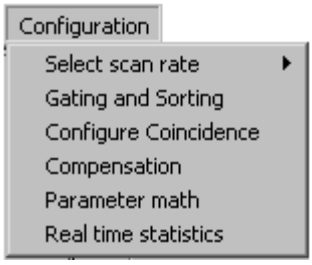
Once chosen the gate/sort polygon will open up to include the entire gate or sort dot plot.

NOTE: Once a GATE region is drawn only events within that GATE region will be displayed on the SORT dot plot. It may be necessary to draw different gate and sort regions to ensure you are including the important data to be displayed on sort dot plot.

TIP!

Regions are automatically saved when Acquired data is stored; however, it is advisable to save template settings every time changes are made. To do this, choose SAVE AS under the FILE drop down menu and designate an appropriate location and file name.

7.14.6 CONFIGURATION



SELECT SCAN RATE

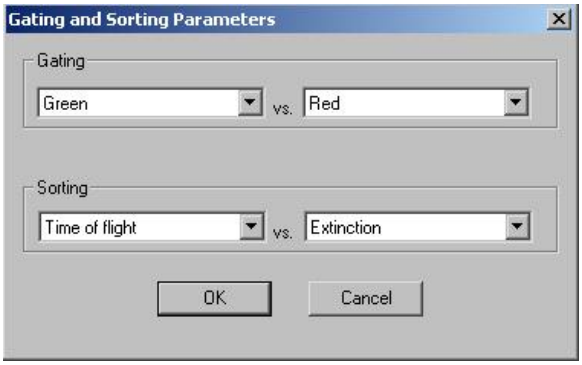
Scan rate refers to the clock speed of data capture or the rate at which the processor cycles through each parameter to capture one cycle of data. Once an object's signal is detected above the threshold value, the processor begins collecting a data point for each parameter channel, cycling through each successively until the object's signal falls below the threshold value. The scan rate is in essence the time the user sets for one cycle of data collection for each parameter. The fastest rate is a 5 MHz setting in which it takes 0.2 µs for the processor to capture one cycle of parameter information while the slowest setting, 156 KHz, captures one cycle of data over 6.4 µs.

For applications not requiring Profiler option, there is little affect on data generation other than an enhanced sensitivity for small object detection. However, higher scan rates require more processing time and may affect whether the system can determine a sorting decision in enough time to dispense an object.

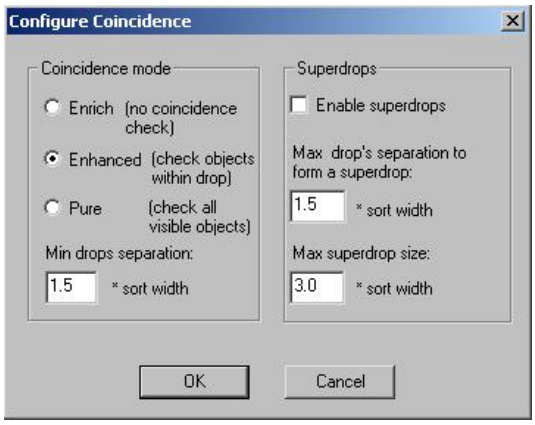
The default setting is 2.5 MHz, but user can change the setting any time system is not sampling in Data collection mode. Also note that the stored txt file of data contains a column denoting which scan rate was used during data acquisition.

GATING AND SORTING

User can select from GATE and SORT pull down menus which parameters' data to display in gate and sort dot plots.

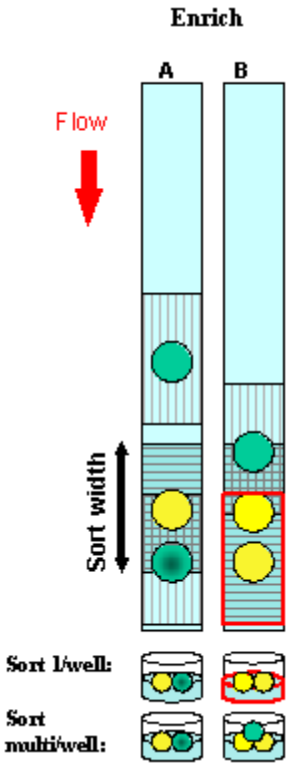


CONFIGURE COINCIDENCE



Coincidence can be described as two objects occurring in the sample stream close enough in proximity to each other that their signals may interfere with each other preventing accurate data collection. The system uses a narrow window of detection in which to determine the object's parameter information as well as make a sort decision about the sample object. If two objects are close enough in the sample stream, the system risks collecting incorrect information about the two objects or dispensing one or both incorrectly.
Consider that with some samples, it may be necessary to dispense only few objects, requiring that a single event be sorted to a single well. Other samples may have a large number of debris particles, each particle possibly close enough to a sample object to create a coincidental event that prevents the object from being sorted. For these reasons the system makes use of different user selected modes for considering object coincidence. See the figure below which serves as a visual display of the mechanism the system uses to determine coincidence.

ENRICH MODE—no coincidence check
In Enrich mode, the system makes no coincidence check. The system only analyzes objects for properties fulfilling sort criteria.

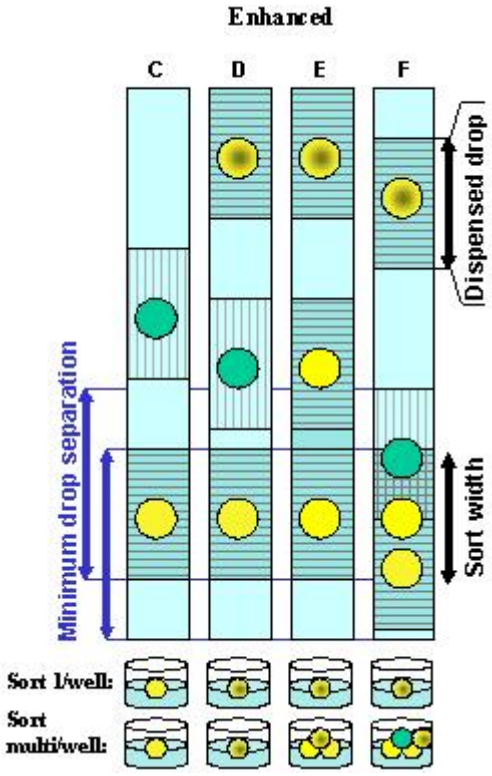


Single object sort:
When it detects a sortable object, it will dispense the minimum drop containing the sortable object, the sort width of the single sortable object. SEE THE SHADED REGION OF FLOW denoting the user defined OBJECT SORT WIDTH. If the sort width of the sortable object contains any additional objects, those will be dispensed within the object's drop. See column A in the diagram where the yellow and green bead are dispensed in a single drop determined by the sortable, yellow bead. See column B where two yellow beads are dispensed as a single drop determined by the primary sortable yellow bead.

Multiple object sort:
If there are two or more sortable objects close enough in the stream that their individual drop widths overlap, a single drop large enough to span both sort widths will be dispensed. See column B that displays overlapping sort widths of sortable yellow beads. Both yellow beads are dispensed in a larger drop having the size of their overlapping drop widths. If there are contaminating objects within the sort widths of the sortable objects these will also be dispensed within the sorted drop. See column B where a green bead lies within the overlapping sort width of yellow beads and is therefore dispensed. Because contaminating events are not sortable objects, they will not be counted as dispensed events but will be counted in total event count.

ENHANCE MODE—uses closest secondary event and distance of minimum drop separation
Enhance mode determines coincidence of closest object within minimum drop separation. When the system detects a sortable object it looks for the next closest event "secondary event" within the user defined minimum drop separation to determine coincidence.

Single object sort:



If the secondary event's sort width is outside the minimum drop separation, the sortable object will be dispensed. See Enhanced diagram column C where the yellow bead is dispensed because the sample stream within the object's minimum drop separation was free of other events, contained no coincidental events. If any part of the secondary event's sort width lies within the user defined minimum drop separation, the object will NOT be dispensed—secondary event is coincidental to the sortable object. See column D and E where a secondary event's sort width lies partially within the primary yellow bead's minimum drop separation. In this case, the system will not dispense the primary yellow bead because secondary bead is coincidental to a single bead sort.

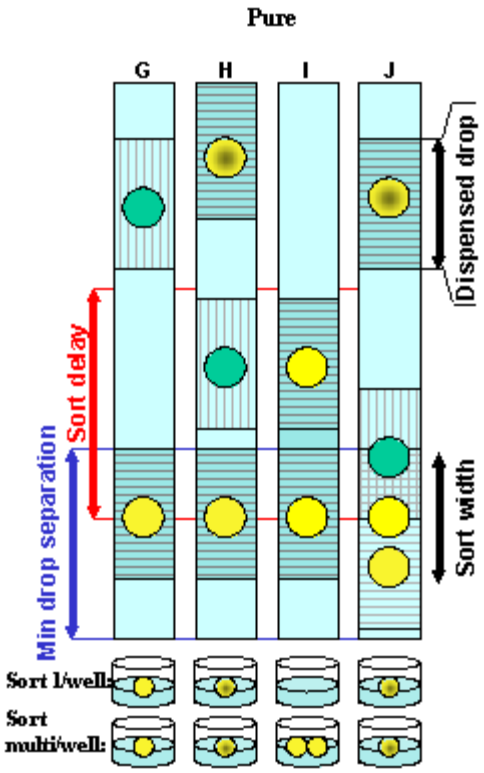
Multiple object sort:
When the system determines that there are two or more sortable objects lined the stream, it will dispense them as efficiently and accurately as possible. If two sortable objects have overlapping sort widths or sort widths that fall within each other's minimum drop separation, the system will dispense the part of the sample stream that contains both sortable objects. See column E in the diagram where closely associated yellow beads cannot be dispensed as a single bead but can be dispensed as a larger drop containing both beads. (This drop includes part of the sample stream between the beads' sort widths)

NOTE: It is important to keep in mind that enhanced mode determines coincidence based on the nearest neighbor to the sortable object even though, on rare occasion, other objects may be in the sort width. In this case coincidence, will be applied to the sortable objects because they are the closest to each other, ignoring the third event for coincidence check. If sort command is more than one per well all three objects will be dispensed together and COPAS *SELECT* registers 2 sorted events. See column F in the diagram where a green bead was dispensed in a single drop containing the two sortable yellow beads. For each sortable object (yellow bead), coincidence was applied to its nearest neighbor (the other yellow bead), excluding the green bead from coincidence analysis. Because the green bead was physically within the sort width of the second yellow bead it was dispensed in the drop containing the sortable yellow beads. It should also be noted that this situation rarely occurs unless the sample is very concentrated or there is a large number of debris particles in the sample itself.

PURE MODE—checks all visible objects within distance of drop delay
In pure mode, once a sortable object is detected the system will check all objects within the sortable objects minimum drop separation and drop delay (distance for object to move into position to be dispensed).

Single object sort:
If a secondary object is detected either within the minimum drop separation in front of the sortable object or within the drop delay behind the sortable object, the sortable object will be rejected as unable to be dispensed singly. In column G a single yellow bead was dispensed because no other events were detected within the minimum drop separation or drop delay, however, column H shows that a green bead was within the drop delay so the yellow bead was rejected. In column I, the secondary yellow bead was within the sort delay of the primary yellow bead therefore it could not be dispensed as a single event. However, the tertiary yellow bead has no coincidental event within minimum drop separation or sort delay; therefore it will be dispensed as a single object.

Multiple object sort command:
When the system encounters two or more sortable objects to dispense it determines coincidence for all visible objects

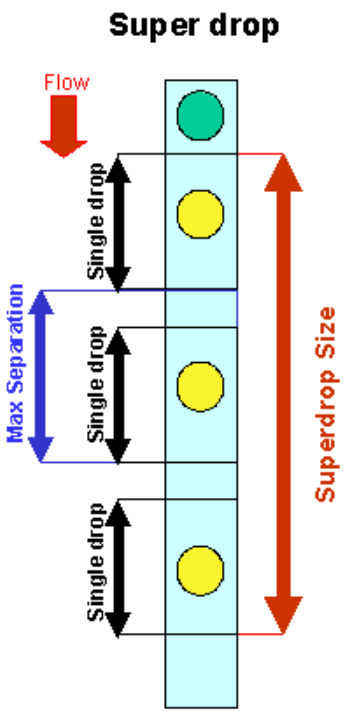


relative to the sortable objects. As long as there are no non-sortable objects within the minimum drop separation and sort delay, the system will dispense all sortable objects as efficiently as possible. In column I, three sortable beads lay within the sort delay of the previous bead. In this case, all three beads can still be dispensed accurately: the first two beads in a single drop containing the sort widths of both beads, and the third bead as a single drop of its sort width. This is only the case if all three are sortable objects. If the third bead was a non-sortable bead, none of the beads could be dispensed because the third bead would be coincidental to the two sortable beads since it would lie within the second bead's sort delay. Moreover, since all visible objects are analyzed for coincidence, the system cannot dispense a contaminating event even if it lies "hidden" within the sort width of sortable objects. In column J, the green bead would be analyzed for coincidence as well as the two other sortable yellow beads. Because it is non-sortable, both yellow beads would be rejected for accurate dispensing.

To set coincidence:

1. Select a Coincidence mode to use.
2. If using Enhanced or Pure modes, set the Minimum drops separation as a function of Sort width. Minimum drops separation should be set to slightly more than the sort width but less than the Sort delay. The system needs to be able to make a sort decision before the object moves past the sorter mechanism. Generally, a value up to 1.5 times the Sort width will be a large enough window to sufficiently analyze objects for coincidence but short enough to make a sort decision by the time they move into position to be dispensed.
3. Click OK to implement the changes.

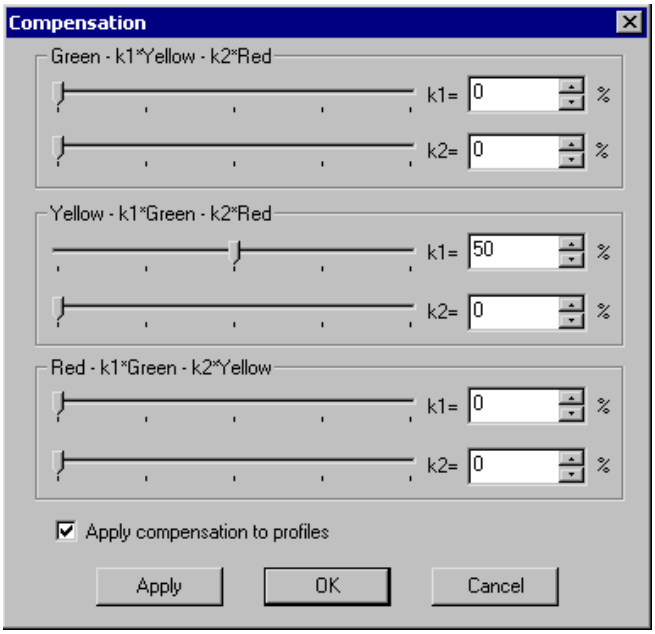
SUPERDROPS

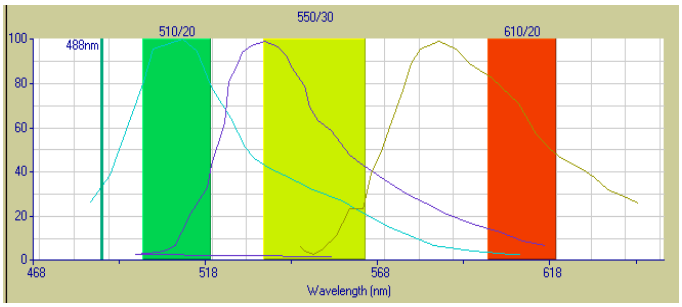


Superdrops is another mechanism the system employs to dispense objects efficiently. Divertor normally turns off and on 6 times to dispense 3 events into separate drops. When super drops are employed, divertor turns off and on only twice to collect the three objects into one large drop containing all three objects. In essence, the diverter valve is turned off to allow the entire sample stream spanning both objects' sort widths and the clear stream separating them to be dispensed as one large drop. Note the system only makes use of this where applicable. If a sort command of 1 per well is initiated superdrops will not be employed.

To use:

1. Open the Configure Coincidence menu item under the CONFIGURATION pull down menu.
2. Check the box to activate Superdrops.
3. Determine the Max drop's separation to form a superdrop as a function of the sort width. This is generally set the same as the Min drops separation (or about 1.5x the sort width).
4. Determine the Max superdrop size as a function of the sort width. Usually 2 x 3 times the sort width. This will allow 2 or 3 normally independent and separate drops to be combined into one larger drop.
5. Click OK to implement the changes.





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

Figure 7.20: Compensation screens with examples for GFP, YFP and DsRed

COMPENSATION

The COPAS instrument uses multiple filters to best capture individual color fluorescence, however, there may can be a considerable amount of fluorescence overlap between the color parameters. The figure above shows the spectral overlap of the color channels in a standard instrument. Clearly there is a broad area of overlap of the emission spectra of the three colors detected, especially in emission of green and yellow fluorescence. The result is that parameter signals often contain some portion of another color's emitted light. This is certainly true for some fluorescent markers. GFP, for example, contains some level of emitted light transmitted by both the green and yellow filters. The result is that the yellow parameter displays some level of green emission. This can affect the data generated for some samples, especially those where both green and yellow fluorescent markers are used. The COPAS instruments employs a feature to compensate for this by allowing the user to subtract a percentage of one color's emitted light from another detector. In the case of GFP, the user can remove a percent of the integral green signal from the total integral signal in the yellow detector. In essence removing green signal from what is detected as yellow. The COPAS software makes this adjustment in real time and used as criteria for a sorting decision.

To use:

1. Open the COMPENSATION dialogue box from the CONFIGURATION pull down menu. The dialogue box lists each color parameter detected as an independent function and the remaining two colors as a variable amount for compensation.
2. To set compensation values, select a color parameter to be adjusted. If removing green from yellow, locate the K1 cursor (green signal) and move to the right to desired percentage to be subtracted from the Yellow channel. The percentage value appears in the box to the right of the sliding bar.
3. Click APPLY then OK and the display will change to that reflecting the compensated values.

NOTE: Compensation values are not stored in the raw data txt or lmd files. Compensation settings will be saved in the stored template, csv file.

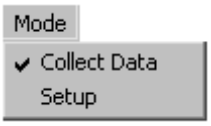
PARAMETER MATH

Allows the user to alter a parameter's data display by mathematical manipulation.

To use:

1. Open the PARAMETER MATH item under the CONFIGURATION pull down menu.
2. Click on ADD to write a new function.
3. Continue applying functions as necessary to make the proper data conversions, giving each an appropriate title.
4. Click OK to implement the changes. At this point the new parameter math titles will appear in the gating and sorting parameter choices in the gating and sorting configuration pull down menu. These can be used for gating and sorting dot plot parameters. NOTE: Stored data in txt and lmd files contain raw data, not the converted parameter functions employed during data azquisition.

7.14.7 MODE



SETUP MODE

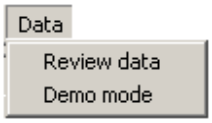
Allows the user to manipulate all user-defined settings while running the sample. User can change PMT's gains, gate/sort regions while the system is running the sample. However, data collected during this mode cannot be stored because settings are not considered uniform throughout data collection.

COLLECT DATA MODE

Prevents the user from making any changes to system conditions while sample is being processed. Once acquisition is completed data can be stored.

NOTE: In Data Collection mode, user settings will be grayed out during data acquisition preventing their modification. If changes need to be made, stop the sample and save what was acquired. You can either make necessary modification to current settings and begin to acquire new data, or change to setup mode to allow you to continue making changes until proper settings are achieved.

7.14.8 DATA



System has the ability to open previously stored data files to re-plot on the software screen. Once opened, user can change dot plot parameters, scale settings, and regions to view previously acquired data.

REVIEW DATA

To Use.

1. While acquisition and sort command is stopped, choose Review Mode under DATA pull down menu. A dialogue box appears prompting the user to select a data file (XXX.bsrt) to review.
2. Click OK to import previously stored data and the template under which data was acquired. Data is plotted on software. Notice a message appears at the bottom of the software indicating review mode is implemented for filename XXX.
3. At this time user can import another template to view data by choosing to Open a file from the FILE menu. (It may be necessary for user to change the scale in order to jog the software to accept the change.
4. User can change parameters for dot plot display, scale settings and regions as they would under normal use.
5. User can save changes to a new template using Save As under the FILE menu.

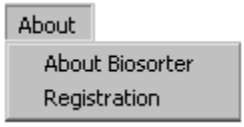
NOTE: Never choose SAVE from the FILE menu, unless you want to permanently alter the original file template. SAVE from the FILE menu does not save the data, only the instrument settings and selected analysis windows.

6. Click ACQUIRE button to return to normal system use. Note however, system will run whatever the current template is. User should import appropriate template file or make changes to return software to appropriate settings.

DEMO MODE

Allows user to reacquire data 2-3 event per second from a previously stored data file. Currently available on Demonstration instruments only.

7.14.9 ABOUT



ABOUT BIOSORTER

Displays the software and firmware revision numbers.

REGISTRATION

Displays the system identification number and registration information including instrument type and software options enabled.

7.15 SFLEX OPTION (Flexible Sorting Templates)

With the SFlex option enabled, the selected well(s) in the SORT GRID AREA can have custom sort counts or regions set. SFlex allows for custom selection of sort windows for dispensing into multiwell plates (24, 48, or 96 well formats). The following parameters can be defined per individual well: number of objects; sorting and dispensing gating parameters; and filling pattern.

8 DATA STORAGE

After data acquisition, data can be stored by clicking on the store button.
In the following text, XXX stands for the filename given by the user.

8.1 SUMMARY FILE

In text format, 'xxx.txt'. This file can be viewed using any text editor or can be exported into a spreadsheet, e.g. Microsoft Excel. The data inside this file is organized in rows and columns, where a row corresponds to data belonging to one object and columns correspond to different parameters and settings for each object.

DESCRIPTION OF COLUMNS IN THE SUMMARY DATA FILE

1. ID
   Sampled objects are given a chronological identifying number starting with 0. This identifier is preserved so a single event can be located.

2. PLATE
   Plates are indexed in chronological order when dispensing into or aspirating from a multiwell plate using a Plate Handler. If dispensing into or aspirating from a plate without a Plate Handler, this column will always contain '1'. If dispensing in a receptacle (Manual Sort) or scanning without dispensing, this column will contain '-1'.

3. ROW
   If dispensing into a plate, this is the well's (numerical) row index, as seen on the plate's template. When acquiring data while plate is not at a well location, a value of 0 will be stored. It is necessary to align the plate correctly to utilize this.

4. COL
   If dispensing into a plate, this is the well's (alphabetical) column index, as seen on the plate's template. When acquiring data while plate is not at a well location, a value of 0 will be stored. It is necessary to align the plate correctly to utilize this.

5. CLOG
   If software detects a clog (based on user defined "Clog Prevention" settings in SETUP menu) while sampling, the file will contain a "Y" otherwise a "N" will be displayed to indicate no problem during sampling.

6. SCAN RATE
   A single scan rate must be used to generate profile data. This rate is displayed as:
   156=156 KHz, 312=312 KHz, 625=625 KHz, 1250= 1.25 MHz, 2500=2.5 MHz, 5000=5 MHz

7. STATUS SORT
   A number representing the sorting status of an event, returned by COPAS *SELECT* board.
   Refer to the following key:
   - 0- Scanning without dispensing
   - 1- Unknown, sort status cannot be retrieved
   - 2- Out of region
   - 3- In enhanced mode: coincidence with following not sortable event
   - 4- Coincidence in pure mode
   - 5- Lost synchronization during acquisition, not sorted
   - 6- Dispensed
   - 7- Dispensed in superdrop
   - 8- In enhanced mode: coincidence with previous not sortable event
   - 9- Sorting decision took too long for event to be dispensed
   - 3n- Sort status equal 30+n, where n is one of the above statuses, indicates that the object was very close to the previous object, and the sorting drop (if n=6) started at least 30 microseconds later than it normally should have.

8. STATUS SELECT
   A Selected status is assigned to each object with respect to its parameter values relative to the gate and sort regions and with respect to its profile characteristics relative to the chosen Profiler sorting configuration.
   0- outside TOF (histogram) gate
   1- outside gating 2-dimensional region (Upper dot plot)
   2- outside sorting 2-dimensional region (lower dot plot if sorting based on 2d regions) or does not satisfy any histogram condition (if sorting based on histograms)
   3—10- does not satisfy a condition for profile peak's height or width
   40– satisfies all sorting conditions

9. TOF
   Time of flight value

10. EXT
    Extinction integral value

11-13. 'Green', 'Yellow', 'Red'
    Integral values for fluorescence on a corresponding channel.

14-25. Contain features only utilized in profiler option. Without profiler enabled all columns will contain 0.

At the bottom left corner of the txt file is a summary of the conditions under which the sample was run. These include the displays of:
   Threshold source, value of threshold = Display of trigger source and trigger threshold value.
   Min TOF = value set as minimum
   Signal gain settings for Ext and each fluorescence channel
   PMT voltage applied to each fluorescence channel
   Data string indicating the location and name of the csv (template) file that the data was generated and stored.

NOTE: Display/Store options affect which data points are stored. Option EVERYTHING stores events from every status select code (all data points). Option STORE GATED excludes events with status select 0 and 1 (outside gate regions). STORE SELECTED excludes events with status select 0, 1, and 2 (outside gate and sort regions).

8.2 A FILE IN FCS FORMAT 'XXX.LMD'

This format is compatible with most flow cytometry software. The file contains all measured integral parameters (extinction, fluorescence, profiling parameter if applicable) and TOF. Scale settings applied during data storage are embedded within this file and cannot be changed during analysis of lmd file.

8.3 A SOFTWARE TEMPLATE FILE 'XXX.CSV'

This format is only compatible with the COPAS software. It is automatically generated upon storage of data. It contains software settings at the time data was generated.

8.4 A FILE IN COPAS FORMAT 'XXX.BSRT'

The bsrt file allows the user to open the file with the template used in the COPAS Software at the time of acquisition. Regions and scaling can be edited (for statistical analysis) or the screen can be printed again..

8.5 A DAT FILE CONTAINING PROFILE INFORMATION 'XXX.DAT'

This format is only compatible with COPAS Profile Reader software. It is a file containing all the profiles of the stored object events. See profile and reader manual for more information.

9 WORK AREA AND FACILITIES REQUIREMENTS

Prior to installation of the COPAS instrument, confirm that the work area and facilities comply with the below specifications.

9.1 WORK AREA REQUIREMENTS

The COPAS and External Laser System(s) require a permanent, open, level, vibration free, working space measuring 2.5 feet (76.2 cm) deep x 7 feet (213.4 cm) wide x 5 feet (152.4 cm) above table height for optimal computer and instrument placement. No other equipment should be placed over the COPAS or External Laser System(s). A COPAS system with the optional plate handler will need an additional space to the left of the COPAS of 3 feet (91.5 cm) deep x 2 feet (61 cm) wide x 2 feet (61 cm) high. The optional Union Biometrica supplied Air Compressor requires approximately 14 inches (36 cm) deep x 18 inches (46 cm) wide x 20 inches (51 cm) high of stable floor space. There must be user access on both ends of the working area.

9.2 ENVIRONMENTAL REQUIREMENTS

This instrument is designed for use at an altitude of up to 2000 meters, in an ambient operating temperature between 15° and 30°C (60° to 85°F) with a relative humidity of 0% to 85% non-condensing, decreasingly linearly to 50% relative humidity at 40°C (104°F). Temperature should not fluctuate more than +/- 1.5 °C from the time of experimental setup through completion or adjustments will need to be made.

The system generates approximately 3800 BTU/Hr. Ensure that adequate ventilation of the system components is provided. It is important that ventilation openings not be blocked while the system is powered ON.

This instrument is rated, per IEC 60529, for installation in an IP00 environment. It is intended that it will be installed in a laboratory environment protected from dust and spray.

9.3 AIR REQUIREMENTS

The input air pressure should be at least 25 PSI but no more than 100 PSI of filtered, at 2 CFM, of non-condensing, water and oil free air. The optional COPAS Air Compressor is available for laboratories that cannot meet these requirements.

CAUTION: Main input regulator must be set to a minimum of 22 PSI.

9.4 ELECTRICAL REQUIREMENTS

Place the COPAS system and components within 6 feet (2 meters) to the power outlets. The COPAS and the External Laser(s) should each be connected to separate, dedicated electrical supply circuits, using the supplied detachable line cords (or CE7/VII approved equivalent detachable cords for Europe). The supply circuits must be compliant with local electrical codes, and must include a dedicated protective ground connection to protect the system. The main supply voltage is not to exceed 10% of the nominal voltage.

Place the instrument so that the detachable line cords remain readily accessible. This is essential to permit use of the line cords as the emergency disconnection devices in case of a fault.

9.4.1 COPAS REQUIREMENTS FOR 120 VAC COUNTRIES

COPAS Instrument, PC & monitor:	100-120 VAC, 15 Amp, 50/60 Hz, single phase on a dedicated line with protective earth ground.
External Laser System:	100-120 VAC, 20 Amp, 50/60 Hz, single phase on a dedicated line with a protective earth ground.
Air Compressor (optional):	100-120 VAC, 15 Amp, 50/60 Hz, single phase on a dedicated line with protective earth ground.

9.4.2 CIRCUIT REQUIREMENTS FOR 230 VAC COUNTRIES

COPAS Instrument, PC & monitor:	220/240 VAC, 10 Amp, 50/60 Hz, on separate dedicated lines with protective earth ground.
External Laser System:	220/240 VAC, 16 Amp, 50/60 Hz, on separate dedicated lines with protective earth ground.
Air Compressor (optional):	220/240 VAC, 10 Amp, 50/60 Hz, on separate dedicated lines with protective earth ground.

9.4.3 FUSE REQUIREMENTS FOR THE COPAS

COPAS Assembly line fuses: (replace as a pair)

Specification:	2.5 A/250V 5 mm x 20 mm, Type M Medium breaking capacity. Approved to: 73/23 EWG, 93/68 EWG, DIN 41571, EN 60127-1
Manufacturer/Part Number:	Schurter #0034-2520
UBI Part number:	067-0002-002

9.4.4 FUSE REQUIREMENTS FOR THE ARGON LASER SYSTEM

Argon Laser assembly:	Present internally on the 310-5010 Laser Interface Printed Circuit Board (replacement performed by authorized personnel only).
120VAC configuration:	
Specification:	200 mA/250V Type T 5 mm x 20 mm, Slow- blow breaking capacity. Approved to: BSI, UL, CSA, SEMKO, IEC 60127-2, and VDE
Manufacturer/Part Number:	Littlefuse #218.200
UBI Part number:	067-0002-009
230VAC configuration:	
Specification:	100 mA/250V Type T 5 mm x 20 mm, Slow- blow breaking capacity. Approved to: BSI, UL, CSA, SEMKO, IEC 60127-2, and VDE
Manufacturer/Part Number:	Littlefuse #218.100
UBI Part number:	067-0002-008

10 REAGENTS

Performance Specifications are only valid with Union Biometrica reagents. DO NOT USE UNAPPROVED SOLUTIONS in the COPAS instruments. The use of unapproved solutions can cause damage to the instrument and will void the warranty. Material Safety Data Sheets are enclosed in every shipment (including supplied reagents) and are also available upon request.

Specialized reagents discussed below are available from Union Biometrica, Inc. Reordering information is located in the Appendices Section of this manual. To determine which Sheath Reagent and Control Particles are recommended for your application, please contact the factory.

10.1 SHEATH REAGENT

Sheath reagent is an aqueous based reagent containing surfactant. The sheath reagent must be compatible with the control particles used and the sample to be analyzed. Two types of sheath reagents have been specially formulated for use with the COPAS *SELECT* instrument. Please review the descriptions of both and select the most appropriate sheath for your application.

300-5070-000, or 300-5070-100 COPAS GP Sheath is recommended for use with all COPAS platforms for all applications EXCEPT *Drosophila* embryo and *D. rerio* applications. For those exceptions, additional sheath formulas have been developed and are detailed below.

335-5070-000, COPAS ESS Sheath has been specially formulated for use with *Drosophila* embryos. At the embryo stage of *Drosophila* development, embryos have certain characteristics requiring an alternate sheath formulation. In order to reduce clumping, it is also recommended that COPAS ESS, part number 335-5075-000, be used.

335-5075-000, COPAS ESS Embryo Sample Solution *Drosophila* embryos are naturally "sticky" and highly susceptible to clumping, particularly once the chorion has been removed. ESS contains a non-toxic surfactant that reduces embryo clumping, thereby reducing risk of system clog.

10.2 CONTROL PARTICLES

Control particles are latex beads that are uniform in size, suspended in a reagent compatible with the sheath reagent and sample to be analyzed. For most COPAS applications, GP 42 Micron Control Particles are recommended for use. Note that ESS 42 micron High Fluorescence Control Particles should be used with the COPAS ESS Sheath reagent (335-5070-000).

310-5071-000, GP 42 micron HF (High Fluorescence) Control Particles are recommended for most applications.

335-5071-000, ESS 42 micron High Fluorescence Control Particles have been specially formulated for use with *Drosophila* embryos.

10.3 SAMPLE

Sample diluents are user selectable. Sample diluents must be compatible with sheath reagent.

NOTE: The sample must be free of debris. Microscopic examination prior to use is highly recommended. Extraneous debris may lead to clogs forming in the flow cell and poor sorting recovery.

10.4 CLEANING REAGENT

Cleaning reagent is an aqueous based reagent containing surfactant. Cleaning reagent should be used for daily maintenance. Do not put Cleaning reagent into the sample cup while sample valve is open. Foaming will occur. Do not leave Cleaning reagent in sample cup or flow cell overnight.

300-5072-000, **COPAS *Cleaning Reagent*** is recommended for use with all COPAS platforms and all applications.

10.5 STERILIZATION SOLUTION

70% Ethanol is used on the COPAS for sterilization procedures and in certain troubleshooting procedures. The manufacturer does not supply ethanol.

10.6 BLEACH

Bleach is used on the COPAS for troubleshooting procedures including breaking up proteins potentially causing a blockage in the flow cell or fluidics. Bleach is also used to clean the internal flow cell. The manufacturer does not supply bleach.

NOTE: Do Not use on external surfaces of optical components

Union Biometrica, Inc. recommends that Clorox™ or Sigma brand bleach be used due to its observed low particulate count. If the bleach is old or a different brand of bleach is used, filtering to remove the large sodium particles is required. When using bleach, use 5% sodium/calcium hypochlorite at a 50% dilution (final concentration should be ~2.5% hypochlorite).

11 INSTALLATION

The COPAS instrument is a Class I laser product containing a Class IIIA laser. Only qualified service personnel should remove the optics assembly covers.

The COPAS External Laser System contains a Class III B laser, which is fiber coupled through a fiber optic. Only qualified service personnel should remove the COPAS External Laser System(s) or the optics assembly covers.

The COPAS will be factory shipped to you in protective packaging and will be installed by a trained Union Biometrica, Inc. representative. Refer to section 9 WORK AREA AND FACILITIES REQUIREMENTS, prior to the arrival of the instrument, and ensure that all the stated requirements have been met.

Refer to the System Interconnection Diagram for the interconnection between the various system components.

A trained Union Biometrica, Inc. representative must install the system. The connection of the Laser fiber optic cable(s) requires special attention and should not be attempted by untrained persons. The Union Biometrica, Inc. representative will also verify the system performance.

Refer to this Operator's Manual, and thoroughly understand the contents prior to operating the instrument.

The attachment of the COPAS to any equipment other than that approved and supported by Union Biometrica, Inc. is not authorized. The lack of functionality of any unauthorized attachment and any resulting damage to the COPAS system is not the responsibility of Union Biometrica, Inc. In addition, if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

In order to prevent the system from overheating, ensure that the installation permits the unrestricted flow of air around the system components.

11.1 MOVING THE COPAS

The COPAS is intended for placement in a permanent location. However, if the COPAS must be moved, a Union Biometrica, Inc. Service Representative must first disconnect the fiber optic cable. Expensive damage to the fiber optic cable(s) may result if it is not removed, protected, and reconnected correctly. Before moving the COPAS, turn the power OFF at the main power switch. Disconnect the power, RS232 connection, and the Laser System cable. If a ReFLx Sampler option is present, disconnect the USB cable. If a Profiler option is present, disconnect the Profiler A/D cable.

Due to the weight of the instrument [40 Kg (88 lbs)], a minimum of two people is required to move the COPAS *(*one person on either side of the instrument). Make sure the new location is free of debris and meets the Working Area requirements as outlined in this Operators Manual and that the path to the new area is free of obstruction. In order to lift the unit safely without tipping, one person should be located on either side of the instrument to lift the unit by placing one hand under the front and one under the rear of the base. Move the COPAS to the desired location. The Service Representative will re-attach the fiber optic cable and other cables, and power up the unit.

To move the Argon Laser System: Due to the weight of the external laser, [33.6 Kg (74 lbs)], a minimum of two people is required to move the unit *(*one person on either side of the instrument). A single person can move any of the Solid State External Lasers. Contact Union Biometrica if you are not sure which laser accompanies your COPAS system. Make sure the new location is free of debris and meets the Working Area requirements as outlined in this Operators Manual and that the path to the new area is free of obstruction. Lift the External Laser System by placing one hand under the front and one under the rear of the base. Move the Laser System to the desired location, plug in the cables and power up the unit.

12 SPECIFICATIONS

12.1 COPAS *SELECT* GENERAL SPECIFICATIONS AND LIMITATIONS

Instrument weight	40 Kg (88 lbs)
External Laser weight	Argon Laser: 33.6 Kg (74 lbs)
Workspace requirement	Refer to section 9 WORK AREA AND FACILITIES REQUIREMENTS Well ventilated
Air pressure	25-100 PSI house air at 2 CFM or Union Biometrica, Inc. supplied air compressor
Ambient temperature	Refer to Section 9 WORK AREA AND FACILITIES REQUIREMENTS
Flow cell size	500 micron
Sample rate	about 2-3 ml/minute
Flow rate	25 ml/minute
Applications	Objects from 100 – 300 micron including: *Drosophila* (fruit fly) embryos to first instar larvae *C. elegans* larvae and adults Large cells such as hepatocytes and adipocytes Beads or particles 20 to about 300 micron diameter Cell clusters up to about 300 micron diameter
Collection device(s)	Bulk 24, 48 and 96 well multiwell plates *96-well Multiwell Plates Recommended for use: Costar (Cat. No. 3598)

For more details, please refer to section 9 WORK AREA AND FACILITIES REQUIREMENTS

12.2 GENERAL PERFORMANCE SPECIFICATIONS

12.2.1 ANALYSIS AND COUNTING RATE

Up to about 250 events per second (maximum).

12.2.2 AUTOMATED DISPENSING FILL TIME FOR 96 WELL MULTIWELL PLATES

105 ±15 seconds, average plate with Coincidence Check software operating and one (1) object per well selected.
180 ± 30 seconds, average per plate with the Coincidence Check software operating and five (5) objects per well selected.

12.2.3 AUTOMATED DISPENSING ACCURACY AND PRECISION

≥ 97.5% accuracy and ≤ 2 coincident events per 96 well multiwell plate.

12.3 FLUID MECHANICAL DESIGN SPECIFICATIONS

12.3.1 SAMPLE CAPACITY

Sample cup maximum capacity is 250 ml (or optional 40 ml).

12.3.2 SAMPLE CONCENTRATION

Adjustable, nominally 250 / ml with a maximum of 500 / ml.

12.3.3 SAMPLE FLOW RATE

Adjustable, standard set by running controls beads at about 10 events/second.

12.3.4 SAMPLE MIXING

The sample cup contains a magnetic stir bar.

12.3.5 SHEATH FLOW RATE

Typically 25 ml per minute at factory set Sheath Pressure and at installation.

12.3.6 CLEANOUT BOTTLE CAPACITY

Cleanout Bottle maximum capacity is 1 liter.

12.3.7 SHEATH BOTTLE CAPACITY

Sheath Bottle maximum capacity is 10 liters.

12.3.8 DIVERTER MECHANISM

Air jet fluid switch activated from signal processing electronics.

12.4 OPTICAL ASSEMBLY DESIGN SPECIFICATIONS

12.4.1 LASER

May include any combination of the following available lasers:
Light stabilized solid stgate laser at 405nm, 488nm, or 640nm wavelengths, operating at approximately 10mW.
Light stabilized 488 nm/514 nm multi-line Argon-ion laser operating at approximately 10 mW.
Light stabilized 670 nm (or 635) semiconductor laser operating at approximately 1 mW.

12.4.2 LASER OPTICS

Up to three laser beams converge at the plane of the flow cell.

12.4.3 FLOW CELL

Square cross-section quartz flow cell with 500 µm square cross-section inner bore.

12.4.4 DETECTOR

Photodiode detector for measuring Extinction (EXT) and Time of Flight (TOF).
Photomultiplier tubes for measuring fluorescence (FLU).

12.4.5 FILTERS

Manual and software selectable fluorescence excitation filters:
Standard configuration for emission filters: Green, 510 nm; Yellow, 545 nm; Red 610 nm. Other emissions filters available from Union Biometrica may be used as well.

12.4.6 AMBIENT LIGHT

Optical assembly is sealed and unaffected by normal room light.

12.5 ELECTRONICS SPECIFICATIONS

12.5.1 PROCESSORS

Three microprocessors controlling X-Y Stage motion, sorting/acquisition, and fluidic control valves.

12.5.2 COMPUTER

IBM compatible PC with color monitor and two serial communication ports.

13 TROUBLESHOOTING

The following categories contain the most common performance problems encountered during operation of the COPAS instrument. Each category contains common symptoms and the basic steps recommended for assessing and correcting the problem. It would be of value to establish a log book that allows users a place to document and track instrument performance.

Some problems may require attention from trained service personnel. Please refer to the contact information in this Operator's Manual if the problem cannot be solved through steps outlined in this section.

CAUTION: DO NOT ATTEMPT SERVICE ON BLUE (EXTERNAL) LASER. IMPROPER SAFETY PRECAUTIONS MAY CAUSE BLINDNESS

Category 1: Pressure

Category 2: Sample Flow

Category 2: Analysis

Category 2: Sorting

Category 2: Instrument

13.1 PRESSURE

If there is no pressure registered in the Pressure Status Field

1. Check house air or pressure pump. Verify it is on and the air tubing is not crimped.
2. Check caps to the sheath container and sample cup. Verify they are tightened securely.
3. Check the sheath inlet line. Verify it is not crimped.
4. Check pressure and sample hoses. Verify that they are properly attached to the sample cup.

If there is low pressure registered in the Pressure Status Field

1. Check house air or pressure pump. Verify the pressure regulator readout is within specification.
2. Check caps to the sheath container and sample cup. Verify that they are tightened securely.
3. Check the sheath inlet line. Verify it is not crimped.
4. Check pressure and sample hoses. Verify that they are properly attached to the sample cup.
5. Adjust the sheath pressure valve if necessary.
6. Take a 1minute sheath volume measurement to ensure correct sheath flow described in Section 4.1 PREPARATION.
7. Adjust the sample pressure valve if necessary.

13.2 SAMPLE FLOW

If there is no sample flow:

1. Check sheath and sample containers. Verify they are filled appropriately.
2. Check sheath and sample pressures. Verify they have reached the specified levels.

3. Check the sample preparation. Verify the concentration of the sample is within specification.
4. Check the sample preparation. Verify there is no debris that may clog the sample valve.
5. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the CLEAN button several times.
    b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
    c. Place a finger over the vent on the sheath bottle cap and then remove it, this action will temporarily increase sheath pressure. Observe pressures while performing this action and be sure not to exceed recommended pressures.
    d. Turn SAMPLE VALVE off and click the sheath valve several times, opening and closing the valve. Often these steps are effective in dislodging the clog and re-starting the flow.
6. Use the stylus to remove any potential clogs in the flow cell and in the case that previous steps failed to remove the clog.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

7. Make sure the Sheath and Sample valves are closed.
8. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.
9. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.
10. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.
11. Remove the stylus and reconnect the sample tubing.
12. Select the CLEAN button 2 – 3 times.
13. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
14. It may be necessary to slightly elevate sample pressure. Increase sample pressure in increments of 0.2 PSI until flow resumes.

If there is spray from the flow stream

1. Check sheath and sample containers. Verify they are filled appropriately.
2. Check sheath and sample pressures. Verify they have reached the specified levels.
3. Check the waste container. Verify it is draining properly.
4. Check the waste tray. Verify it is pushed all the way to the right.
5. Check the outlet nozzle. Verify that liquid has not accumulated around the nozzle. If it has, perform the following:
    a. Remove the waste tray.
    b. Soak up excess liquid around the nozzle with the tip of a paper tissue.
    c. Return the waste tray to position, observe if spray is still present.
6. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the CLEAN button several times.

b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

c. Place a finger over the vent on the sheath bottle cap and then remove it, this action will temporarily increase sheath pressure. Observe pressures while performing this action and be sure not to exceed recommended pressures.

d. Turn SAMPLE VALVE off and click the sheath valve several times, opening and closing the valve. Often these steps are effective in dislodging the clog and re-starting the flow.

7. Use the stylus to remove any potential clogs in the flow cell and in the case that previous steps failed to remove the clog.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

a. **Make sure Sheath and Sample valves are closed.**

b. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.

c. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.

d. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.

e. Remove the stylus and reconnect the sample tubing.

f. Select the CLEAN button 2 – 3 times.

g. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

13.3 ANALYSIS

If control bead TOF C.V.s are out of specification:

1. Check the flow cell for a clog. Clear the flow cell by performing the following:

   a. Click the CLEAN button several times.

   b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

   c. Place a finger over the vent on the sheath bottle cap in order to increase sheath pressure.

   d. Turn SAMPLE VALVE off and click the SHEATH VALVE several times, opening and closing the valve.

2. Use the stylus to remove any potential clogs in the flow cell.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

a. Make sure the Sheath and Sample valves are closed.

b. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.

c. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.

d. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.

e. Remove the stylus and reconnect the sample tubing.

3. Select the CLEAN button 2 – 3 times.
4. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.
5. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.
6. Clean the interior optical path using the Bleach Procedure described in section 6.2 REPLACING THE WASTE PUMP TUBING.

If extraneous events are analyzed (air bubbles or debris):

1. Verify Threshold and Gains. Refer to Section 7.7 ADJUSTING GAIN VALUES.
2. Check sample preparation for purity.
3. Check fluid levels in all bottles.
4. Check all fluid lines for leaks.
5. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.
6. Clean the interior optical path using the Bleach Procedure described in section 6.2 REPLACING THE WASTE PUMP TUBING.

If sample distribution is too broad:

1. Clean the interior optical path using the Bleach Procedure described in section 6.2 REPLACING THE WASTE PUMP TUBING.

13.4 SORTING

If the stage does not return to home position:

1. Click the STOP button twice.
2. Shut down the program.
3. Turn off the instrument power.
4. Wait 15 seconds and restart the instrument.
5. Reopen the program by selecting the Union Biometrica software icon.

If the droplets do not fall within the wells:

1. Verify stage alignment according to section 4.6.3 ALIGN STAGE.
2. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the CLEAN button several times.

b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

c. Place a finger over the vent on the sheath bottle cap in order to increase the sheath pressure.

d. Turn SAMPLE VALVE off and click the SHEATH VALVE several times, opening and closing the valve.

3. Use the stylus to remove any potential clogs in the flow cell.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

a. Make sure the Sheath and Sample valves are closed

b. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.

c. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.

d. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.

e. Remove the stylus and reconnect the sample tubing.

4. Select the CLEAN button 2 – 3 times.

5. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin

6. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.

If there is an insufficient number of wells filled:

1. Adjust the SORT DELAY until the number of events desired is obtained.

2. Check the flow cell for a clog. Clear the flow cell by performing the following:

a. Click the CLEAN button several times.

b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin.

c. Place a finger over the vent on the sheath bottle cap.

d. Turn SAMPLE VALVE off and click the SHEATH VALVE several times, opening and closing the valve.

e. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.

3. Use the stylus to remove any potential clogs in the flow cell.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

a. Make sure the Sheath and Sample valves are closed

b. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.

c. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.

d. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.

e. Remove the stylus and reconnect the sample tubing.

4. Select the Clean button 2 – 3 times.
5. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin
6. Clean the interior optical path using the Bleach Procedure described in section 0 REPLACING THE WASTE PUMP TUBING.

If there are multiple objects in wells filled:

1. Verify the COINCIDENCE Method reads Enhanced or Pure.
2. Adjust the SORT WIDTH using the field provided. Refer to section 4.6.4 VERIFY SORTING ACCURACY.
3. Adjust the SORT DELAY using the field provided. Refer section 4.6.4 VERIFY SORTING ACCURACY.
4. Check the sample preparation. Verify the concentration of the sample is within specification.
5. Adjust the THRESHOLD. Refer to section 7.7 ADJUSTING GAIN VALUES.
6. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the CLEAN button several times.
    b. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow. When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin
    c. Place a finger over the vent on the sheath bottle cap.
    d. Turn SAMPLE VALVE off and click the SHEATH VALVE several times, opening and closing the valve.
    e. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.
7. Use the stylus to remove any potential clogs in the flow cell.

NOTE: Always inspect stylus prior to inserting into the flow cell as damage may result if the stylus is bent or cut.

NOTE: Always make sure Sheath and Sample valves are closed before using the stylus.

a. Make sure the Sheath and Sample valves are closed

b. Disconnect sample tubing from above the flow cell, taking care to not pull on the flow cell.

c. Insert the stylus into the flow cell, while gently twisting. If resistance is felt STOP, lift the stylus approximately 2 mm and insert the stylus again. Repeat this as necessary until the stylus exits the flow cell without any resistance.

d. Raise and lower the stylus a few times while making small circles with the stylus at the bottom of the flow cell.

e. Remove the stylus and reconnect the sample tubing.

8. Select the CLEAN button 2 – 3 times.
9. Prime the flow cell by selecting PRIME FLOW CELL from the MAINTENANCE menu item, under the Tools drop-down menu. Priming of the flow cell with Sheath fluid removes air bubbles that might prevent stable sample flow.

When the software determines that this step is required at least one complete prime cycle must be completed before acquisition or sorting can begin

10. Clean the interior optical path using the Bleach Procedure described in section6.2 REPLACING THE WASTE PUMP TUBING.

13.5 INSTRUMENT

If there is no power to the instrument"

1. Verify the instrument power supply is connected and plugged in.
2. Verify the instrument IO switch is turned ON.
3. Verify the fuses are operational. Replace fuses as follows:
    a. Turn the unit OFF by switching the main power switch, located on the left side of the instrument, to the O position.
    b. Disconnect the power cord.
    c. Depress the tab on the fuse drawer, located on the left side of the instrument, and remove the drawer containing the fuses.
    d. Discard the old fuses.
    e. Replace the fuses according to section 9.4 ELECTRICAL REQUIREMENTS.
    f. Replace the fuse drawer.
    g. Connect the power cord.
    h. Turn the unit ON by switching the main power switch to the "I" position.

If there is pooling under the waste tray:

1. Replace the waste pump tubing according to section 0 .

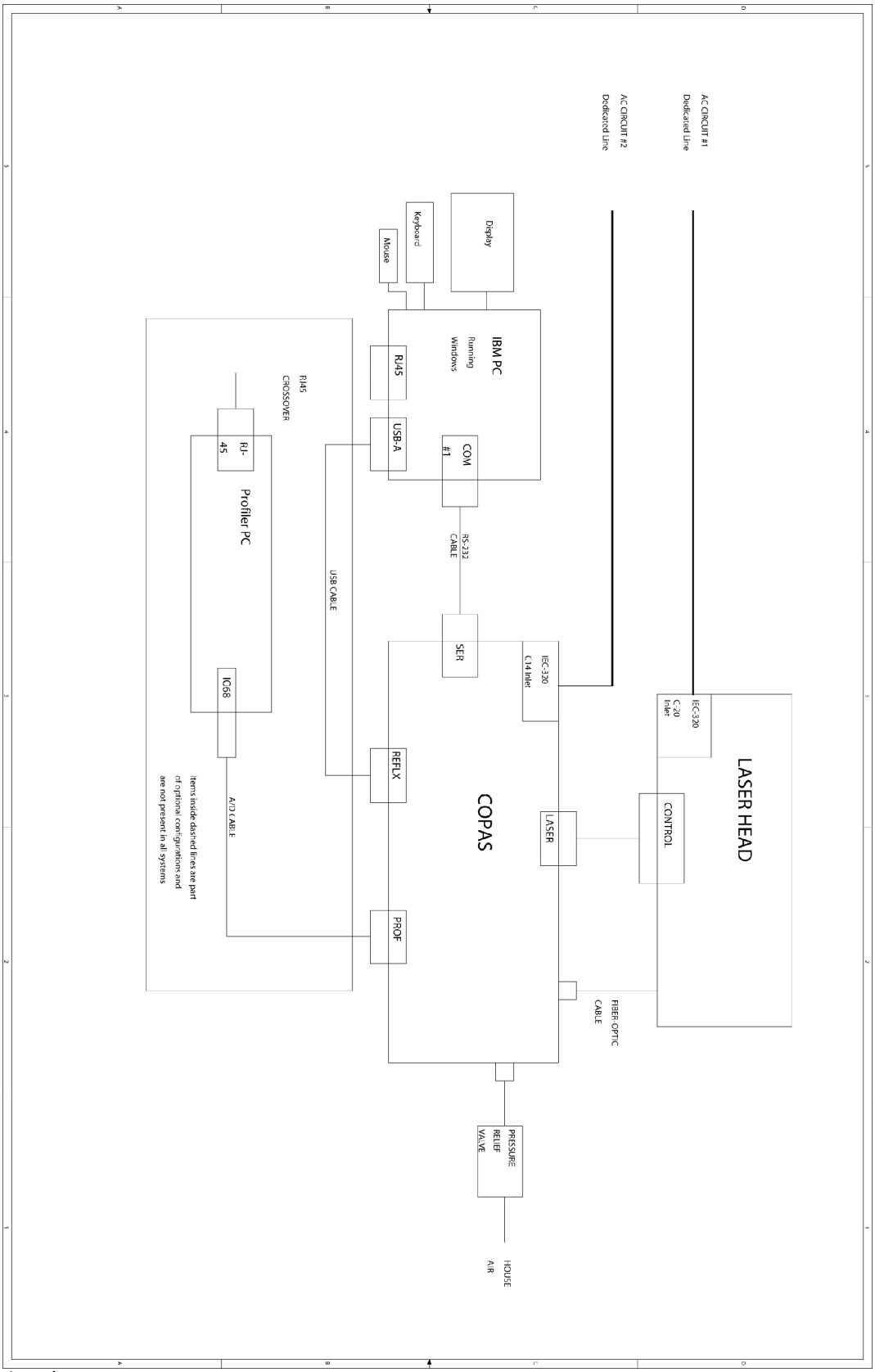
For any other questions or problems call:

US – Global Headquarters
Union Biometrica, Inc.
Tel: 508-893-3115
Fax: 508-893-8044
Email: sales@unionbio.com

Europe
Union Biometrica N.V.
Tel: +32 14 570 628
Fax: +32 14 570 629
Email: sales@unionbio.com

14 APPENDICES

14.1 APPENDIX A: Schematic of COPAS System



14.2 APPENDIX B: Daily Performance Log Sheet

Date	Control Particle Lot Number	Sheath Pressure	Sample pressure	TOF C.V.	TOF Mean Channel

14.3 APPENDIX C: Maintenance Log Sheet

DAILY

Cleaning Reagent Lot No.	Date	Time	Performed By

SHORT/LONG TERM SHUTDOWN

Deionized Water	Date	Time	Performed By

14.4 APPENDIX D: Maintenance Log Sheet

Ethanol Solution Lot No.	Date	Time	Performed By

14.5 APPENDIX E: Maintenance Log Sheet

Maintenance Performed	Date Performed	Performed By

14.6 APPENDIX F: Troubleshooting Log Sheet

Date	Symptom	Troubleshooting Performed	Initials

14.7 APPENDIX G: Consumables Re-Order Form

CONSUMABLES RE-ORDER FORM
FOR COPAS™ & BioSorter™ INSTRUMENTS

Last revision: June 2008

UNION BIOMETRICA

Please send completed form to: **FAX: +1-508-893-8044 or EMAIL: orders@unionbio.com**
Questions? **Call +1-508-893-3115 x284**

Contact & Ship To:
Contact Name: _____
Account Name: _____
Address: _____
_____
City/St./ Zip: _____
Phone: _____
Fax: _____
Email: _____

Bill To: (if different)
Contact Name: _____
Account Name: _____
Address: _____
_____
City/St./Zip: _____
Phone: _____
Fax: _____
Email: _____

PURCHASE ORDER NUMBER: _____
CREDIT CARD NUMBER: _____ **Expiration Date:** _____
☐ Master Card ☐ Visa ☐ American Express
NAME AS IT APPEARS ON CARD: _____
MAILING ADDRESS IF DIFFERENT THAN BILL TO: _____
_____

Qty	Part Number	Description	List Price 1-9 units	List Price 10+ units
____	300-5070-100	COPAS GP (General Purpose) Sheath Concentrate (40mL bottle, makes 10L)	**Contact Union Biometrica for Current Pricing**	
____	310-5071-000	GP 42 micron Control Particles (1L bottle)		
____	300-5072-000	COPAS Cleaning Reagent, All Platforms (1L bottle)		
____	335-5070-000	COPAS ESS Sheath (20L container)		
____	335-5075-000	COPAS ESS (Embryo Sample Sol.) (1L bottle)		
____	335-5071-000	ESS 42 micron Control Particles (1L bottle)		
____	300-5100-000	COPAS Cell Sheath Concentrate (1L bottle, makes 10L)		
____	300-5101-000	COPAS Worm Sheath Concentrate (1L bottle, makes 10L)		
____	300-5102-000	Cell & Worm 42 micron Control Particles (1L bottle)		
____	370-5070-100	COPAS Z-Sheath Concentrate (2x1L bottles, makes 100L)		
____	370-5071-000	Z 500 micron Control Particles (1L bottle)		
____	360-5072-000	50,100,200,300,400,500um - Six Bead Mixed Control Particles, NIST Traceable beads (1L bottle)		
____	360-5072-100	50,100,200,300um - Four Bead Mixed Control Particles, NIST Traceable beads (1L bottle)		
____	340-5014-000	ReFLx Filter 10 pack		
____	350-5031-000	Sheath Bottle Filter assembly with appropriate tubing and fittings.		
____	350-5032-000	Cleanout / ReFlx Bottle Filter assembly with appropriate tubing and fittings.		
____	350-5033-000	Air Line Filter assembly with appropriate tubing and fittings.		
____	111-0125-001	Waste Pump Tubing, 1/8" ID x 1/4" OD for older Biosort & Select instruments (sold by the foot)		
____	112-0250-001	Waste Pump Tubing, 1/4" ID x 3/8" OD (sold by the foot)		
____	111-1301-000	Waste Pump Tubing for XL instruments, small tubing. (sold by the foot)		
____	111-1302-000	Waste Pump Tubing for XL instruments, large tubing. (sold by the foot)		

Prices subject to change without prior notice. Local taxes and shipping charges will be applied to order total and will be reflected on the invoice. An order confirmation will be faxed or emailed to you.

SPECIAL INSTRUCTIONS (Please indicate shipping preference): _____