UNION BIOMETRICA

Large Particle Flow Cytometry

BioSorter® INSTRUMENT

WITH FlowPilot™ or FlowPilot-Pro™ Software

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

OPERATOR'S MANUAL

Rev. 2.0

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UNION BIOMETRICA
Large Particle Flow Cytometry

DECLARATION OF CONFORMITY

Standards to which Conformity is Declared

Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use:
• IEC 61010-1:2001, EN 61010-1:2001, including Group and National Differences as they apply for Australia, Canada, the United States, and Korea

Safety of Laser Products:
• IEC 60825-1:2007 (2nd Edition), EN 60825-1:2007 (2nd Edition),

EMC Emissions:
• ICES-003 Issue 4 Class A Digital Apparatus emissions requirements (Canada)
• FCC 47 CFR Part 15 Class A emissions requirements (USA)
• EN 55011: 2007/A2:2007 Group 1 Class A ISM emissions requirements (EU)
• EN 61000-3-2:2006 Limits for harmonic current emissions (equipment input current up 10 and including 16A per phase)
• EN 61000-3-3:1995/A1:2001/A2:2005 Limitation of voltage fluctuations and flicker in low-voltage supply systems for equipment with rated current up to and including 16 A

EMC Emissions and Immunity:
• EN 61326:2006 EMC requirements for Electrical equipment for measurement, control and laboratory use - General Use

Manufacturer's Name	Union Biometrica, Inc.
Manufacturer's Address	84 October Hill Road Holliston, MA 01746 USA
Type of Equipment	BioSorter ® System
Model Number	600-5000-000
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 BIOSORTER INSTRUMENT OVERVIEW

The BioSorter instrument 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 plates for high throughput screening. The BioSorter 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 BioSorter instrument handles objects ranging from 20 - 1,500 microns utilizing a specially engineered Fluidics and Optics Core Assembly (FOCA) containing a quartz flow cell optimized for a subset of the size range.

1.2 SORTING PARAMETERS

The instrument utilizing FlowPilot software measures five parameters of information of each object. FlowPilot-Pro has active the Profiling feature adding an additional 12 parameters of information.

FlowPilot Standard:

- 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

FlowPilot-PRO additional parameters for each profiled channel (extinction, and 3 colors of fluorescence)

- Parameter peak intensity
- Parameter peak width
- Parameter peak count

BioSorter 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 the BioSorter instrument is designed on the basic principles of flow cytometry, the BioSorter 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 BioSorter 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.

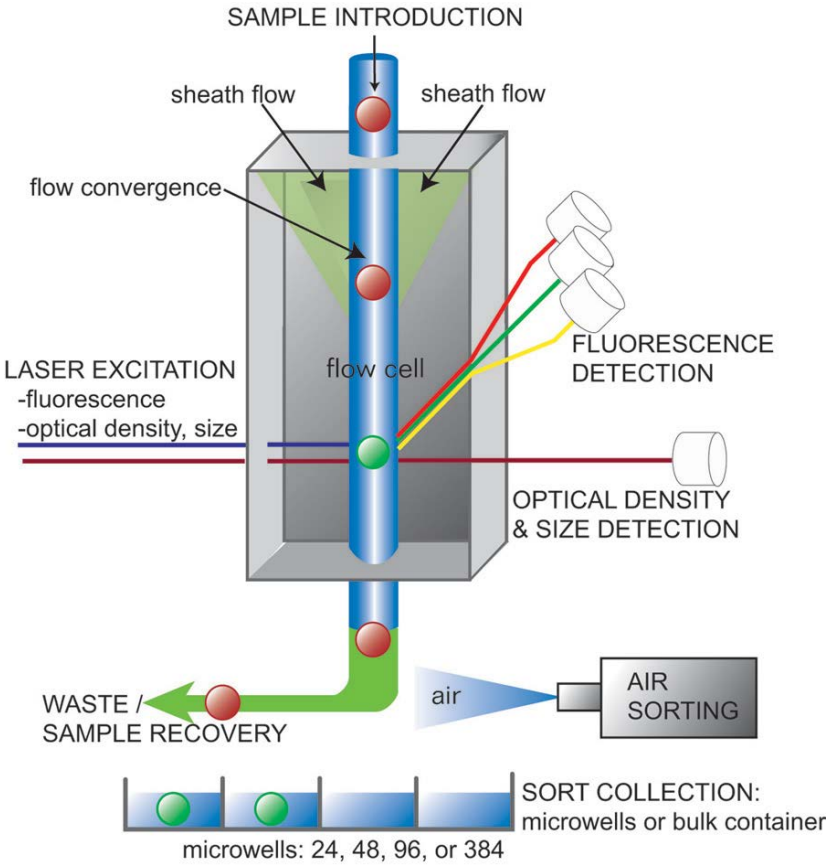

Figure 1 BioSorter Principles of Analysis and Sorting Schematic

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 lasers 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

A PC computer, such as an IBM compatible, preloaded with the FlowPilot-Pro 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 FlowPilot-Pro software.

1.5 DATA OUTPUT AND ARCHIVING

Raw data collected from the analysis is stored both as a text file and in a format compatible with most flow cytometry software, such as WinMDI or FCS Express. The FlowPilot 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 BioSorter user interface (bxrt file).

1.6 AUTOMATION

Instruments are equipped with an X-Y-Z stage and software that can control the dispensing of objects into any user defined receptacle or multi-well plate. Throughput may be further increased with the addition of a robotic-arm for plate handling, specifically for loading and unloading multi-well plates onto the system stage.

1.7 CONTACT INFORMATION

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

US Global Headquarters

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1.8 PATENT & TRADEMARK INFORMATION:

This instrument is covered by the following patents and trademarks:

U.S. Patent No. 6,400,453
U.S. Patent No. 6,657,713
U.S. Patent No. 7,116,407
International WO0036396

BioSorter is a registered trademark of Union Biometrica, Inc.

1.9 SYMBOLS AND NOTES USED IN THIS OPERATOR'S MANUAL

This manual contains operation, maintenance and safety information for Union Biometrica's BioSorter® Instrument.

The BioSorter Instrument is comprised of the Main Instrument, Fluid Caddy and Computer. This term is used in reference to the combination of these components.

The BioSorter instrument 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.

Read this manual before operating the instrument. Other than procedures discussed in this manual, there are no electrical, optical or mechanical adjustments or serviceable components.

Use of the BioSorter instrument in a manner not specified by Union Biometrica may void the BioSorter instrument warranty and impair the protection provided by the equipment.

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 BioSorter 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 BioSorter Software screen

1.9. Symboles et de remarques UTILISÉS DANS CE MANUEL D'UTILISATION

Ce manuel contient des informations de fonctionnement, l'entretien et la sécurité pour l' instrument BioSorter ® de l'Union Biometrica.

L'instrument BioSorter est composé de l'instrument principal, le récipient fluide et l'ordinateur. Ce terme est utilisé en référence à la combinaison de ces pièces.

L'instrument BioSorter doit être utilisé uniquement par le personnel de laboratoire qualifié. L'utilisation de cet instrument par un opérateur inexpérimenté peut entraîner des dommages au produit ou des dommages corporels à l'opérateur.

Lisez ce manuel avant d'utiliser l'instrument. Hormis procédures décrites dans ce manuel, il n'existe aucun réglage électrique, optique ou mécanique ou des pièces utiles.

L'utilisation de l'instrument BioSorter d'une manière non spécifiée par l'Union Biometrica peut annuler la garantie d'instrument BioSorter et affaiblir la protection assurée par l'équipement.

Ce symbole tout au long dece manuel indique à l'utilisateur, qu'ildevrait tenir compte des informations présentées et lire attentivement la section avant d'utiliser l'instrument BioSorter.

Remarque : Ce terme est utilisé tout au long de ce document pour souligner les informations importantes d'exploitation ou les informations importantes pour l'opérateur.

Type Bold : Ce type de police est utilisé tout au long de ce document pour mettre en évidence des informations d'usage importantes pour l'opérateur.

MAJUSCULES : Ce style de caractères se reporte à un élément du menu dans l'écran du logiciel de Biosorter.

2 SAFETY

2.1 SAFETY HAZARDS

The BioSorter instrument was evaluated and conforms to the international safety standards IEC 61010-1:2001, EN 61010-1:2001, and IEC 60825-1:2007. This instrument is classified as a CLASS 1 LASER PRODUCT.

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.

In the case of an emergency, the AC power cord may be unplugged from the instrument in order to remove all power from the BioSorter instrument device. 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.

2.2 SAFETY SYMBOLS LOCATED ON THE BIOSORT INSTRUMENT

All labels located on the BioSorter instrument indicate that a hazard may exist and the operator should carefully read this Operator's Manual for more detail. Do not remove any part of any label on the BioSorter instrument.

 This symbol is intended to alert the operator to the presence of important operating and maintenance instructions.	 This symbol is intended to alert the operator to the danger of exposure to hazardous visible and invisible laser radiation.
 This symbol 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.	 This symbol is intended to alert the operator to the presence of dangerous voltages that may be of sufficient magnitude to constitute a risk of electric shock.

2.3 SAFE OPERATION

All covers, access panels and doors that require tools to remove or open must be in place for the safe operation of this instrument. Do not operate with any of these removed or open. Only the FOCA access cover (front left cover) and the sample recovery access panel (left side) may be open by an operator.

The BioSorter uses lasers and is a CLASS 1 laser product as specified by the United States National Center for Device and Radiological Health (CDRH). No laser hazards exist as long as the safety covers are in place.

DO NOT OPERATE THIS INSTRUMENT WITH THE SAFETY COVERS REMOVED.

CAUTION - VISIBLE AND INVISIBLE CLASS 3B LASER RADIATION MAY BE EMITTED WHEN THE SAFETY COVERS ARE REMOVED. Only Union Biometrica qualified service personnel should remove any of the safety covers.

2.4 ELECTRICAL SAFETY & REQUIREMENTS

A dedicated power cord and power strip is used on this system. The power strip is to be used only with BioSorter instrument components. Do not plug anything other than BioSorter instrument components into this dedicated power strip.

The BioSorter instrument does not contain any user-serviceable electrical components. Do not remove any of the safety covers. Risk of shock due to hazardous voltages exists if the instrument is run with the safety covers removed.

CIRCUIT REQUIREMENTS FOR 120 VAC COUNTRIES

BioSorter Instrument:	100-120VAC, 15-20 Amp, 50/60 HZ, single phase with protective earth ground.
Air Compressor (optional):	100-120VAC, 15-20 Amp, 50/60 HZ, single phase with protective earth ground.

CIRCUIT REQUIREMENTS FOR 230 VAC COUNTRIES

BioSorter Instrument:	220/240VAC, 10-16 Amp, 50/60 HZ, with protective earth ground.
Air Compressor (optional):	220/240VAC, 10-16 Amp, 50/60 HZ, with protective earth ground.

FUSE REQUIREMENTS FOR THE BIOSORTER INSTRUMENT

BioSorter Instrument line fuses should not be substituted. Replace with Union Biometrica parts only.

Specification:	5.0 A / 250V, 5 x 20 mm, Slow-Blow Fuse. Approved to: IEC 60127-2
Union Biometrica part number:	067-0002-015 (two required for 230 VAC countries)

2. Sécurité

2.1. Les risques de sécurité

L'instrument BioSorter a été évalué et est conforme aux normes internationales de sécurité IEC 61010-1: 2001, EN 61010 - 1: 2001 et IEC 60825 - 1:2007. Cet instrument est classé comme un produit LASER de classe 1.

Aucun liquide inflammable pouvant entraîner la propagation du feu dans des conditions normales ou en cas de conditions de panne unique ne sont contenus dans ou spécifiés pour l'utilisation de cet instrument.

Aucune substance dangereuse n'est requise ou spécifiées pour l'utilisation de cet équipement, et aucune substance dangereusen'est produite par son utilisation.

Les réactifs fournis par l'Union Biometrica, Inc. pour l'utilisation de cet équipement sont livrés avec la fiche signalétique appropriée (FTSS) pour les informations liées à la sécurité.

Si des produits chimiques non fournis par l'Union Biometrica, Inc. sont utilisés conjointement avec cet instrument, veillez à observer les pratiques de laboratoire en toute sécurité et se reporter à la fiche signalétique appropriée (FTSS) pour les instructions liées à la sécurité.

Si l'équipement n'est pas utilisé d'une manière spécifiée par le fabricant, la protection assurée par l'équipement peut être affectée.

En cas d'urgence, le fil électrique peut être débranché de l'instrument afin de retirer tout courant de l'instrument BioSorter. Reportez-vous à la section labels d'avertissement ci-dessous et assurez-vous de comprendre les dangers rréférencés par les étiquettes.

Afin d'empêcher le système de surchauffe, assurez-vous que l'installation permette la circulation sans restriction de l'air autour des composants du système.

2.2. Symboles de sécurité SITUÉS SUR L'INSTRUMENT COPAS

Toutes les étiquettes situées sur l'instrument BioSorter indiquent qu'un danger peut exister et que l'opérateur doit lire attentivement ce manuel d'utilisation pour plus de détails. N'ôtez aucune partie d'aucune étiquette Ne pas enlever tout ou partie de l'étiquette de l'instrument BioSorter.

 Ce symbole est destiné à alerter l'opérateur de la présence d'instructions importantes d'entretien et de fonctionnement.	 Ce symbole est destiné à alerter l'opérateur du risque d'exposition aux rayonnements dangereux visibles et invisibles du laser.
 Ce symbole indique un risque de pincement où les utilisateurs devraient prendre soin de ne pas placer les mains ou d'autres objets dans la zone à risque afin d'éviter des blessures ou des dommages au système.	 Ce symbole est destiné à alerter l'opérateur de la présence de tensions dangereuses qui peuvent être d'une ampleur suffisante pour constituer un risque de choc électrique.

2.3. Opérations sans danger

Tous les couvercles, les panneaux d'accès et les portes qui requièrent des outils permettant de les retirer ou de les ouvrir doivent être mis en place pour l'utilisation en tout sécurité de cet instrument. Ne travaillez pas lorsque l'un d'eux est supprimé ou ouvert. Seul le couvercle d'accès FOCA (couvercle avant gauche) et le panneau d'accès de la récupération de l'échantillon (côté gauche) peuvent être ouvertes par un opérateur.

Le BioSorter utilise des lasers et est une PRODUIT LASER CLASSE 1comme spécifié par le Centre National des États-Unis pour les dispositifs et la santéradiologique (CDRH). Aucun risque de laser n'existe tant que les couvercles de sécurité sont en place. **N'UTILISEZ PAS CET INSTRUMENT SANS LES COUVERCLES DE SÉCURITÉ.**

ATTENTION – des radiations laser classe 3B visibles et invisibles peuvent être émise lorsque les couvercles de sécurité sont ôtés. Seul le personnel de service qualifié de l'Union Biometrica ôter l'un des couvercles de sécurité.

2.4 Sécurité électrique et conditions requises

un cordon et une languette d'alimentation/de courant appropriés sont utilisés sur ce système. La languette d'alimentation/de courant ne peut être utilisée qu' avec les composants de linstrument BioSorter. Ne branchez que des composants de l'instrument BioSorter dans cette languette dédiée.

L'instrument BioSorter ne contient pas de composants électriques exploitables par l'utilisateur. N'ôtez aucun des couvercles de sécurité. Si l'instrument est utilisé avec les couvercles de sécurité ôtés il y a risque de choc.

EXIGENCES DE CIRCUIT REQUISES POUR LES PAYS 120 VAC

BioSorter Instrument :	100-120VAC, 15-20 A, 50/60 HZ, monophase avec la terre protectrice
compresseur d'air (facultatif):	100-120VAC, 15-20 A, 50/60 HZ, monophase avec la terre protectrice.

EXIGENCES DE CIRCUIT REQUISES POUR PAYS 230 VAC

BioSorter Instrument :	240 220/VCA, A 10-16, 50/60 HZ, avec terre protectrice.
compresseur d'air (facultatif):	240 220/VCA, A 10-16, 50/60 HZ, avec la terre protectrice.

EXIGENCES DE FUSIBLES POUR L'INSTRUMENT BIOSORTER

Les fusibels de l'instrument BioSorter ne doivent pas être remplacés. Remplacez les uniquement par des pièces de l'Union Biometrica.

Spécification : 5.0 A / 250 v, 5 x 20 mm, Time-retard fuse. Approuvé à: IEC 60127-2.

Numéro de référence de Biometrica Union : 067-0002-015 (deux requis pour pays 230 VAC)

3 INSTALLATION

The BioSorter instrument is a Class 1 Laser Product containing Class 3B laser(s). Only qualified service personnel should remove the optics assembly covers.

- The BioSorter instrument will be installed by a trained Union Biometrica representative. Refer to the section of this manual, 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 instrument components.
- A trained Union Biometrica representative must install the system. This person will also verify the system performance.
- Read this Operator's Manual and thoroughly understand the contents prior to operating the instrument.
- Do not modify the BioSorter instrument. Do not attach any equipment other than those approved and supported by Union Biometrica. Damage to the BioSorter instrument resulting from non-approved modifications or devices is not the responsibility of Union Biometrica. If the equipment is used in a manner not specified by Union Biometrica, 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.

3.1 MOVING THE BIOSORTER INSTRUMENT

- The BioSorter instrument is intended for placement in a permanent location. However, if the BioSorter instrument must be moved, turn the power OFF at the main power switch and disconnect the power cord.
- Due to the weight of the instrument a minimum of four people is required to move the BioSorter 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.

3. INSTALLATION

L'instrument BioSorter est un Produit Laser de Classe 1contenant un (des)laser(s) de Classe 3B. Seul le personnel de service qualifié peut ôter les couvercles d'assemblage optique.

- L'instrument BioSorter sera installé par un représentant qualifié de L'Union Biometrica. Reportez-vous à la section de ce manuel, la zone de travail et les conditions d'établissement, avant l'arrivée de l'instrument et de assurez-vous que toutes les exigences indiquées ont été remplies.
- Reportez-vous au schéma d'Interconnexion du Système pour l'interconnexion entre les différentes pièces de l'instrument.
- Un représentant qualifié d'Union Biometrica doit installer le système. Cette personne sera également vérifier les performances du système.
- Lisez attentivement ce manuel d'utilisation et veillez à comprendre le contenu avant d'utiliser l'instrument.
- Ne modifiez pas l'instrument BioSorter. N'y joignez aucun matériel autres qu'approuvé et pris en charge par l'Union Biometrica. Des dommages à l' instrument BioSorter résultant de modifications ou d'applications non agréées n'est pas la responsabilité de l'Union Biometrica. Si l'équipement est utilisé d'une manière non spécifiée par l'Union Biometrica, la protection assurée par l'équipement peut être affectée.
- Afin d'empêcher le système de surchauffer, assurez-vous que l'installation permette la circulation sans restriction de l'air autour des composants du système.

3.1. DÉMÉNAGER L'INSTRUMENT BioSorter

- L'instrument BioSorter est destiné à être placé dans un endroit fixe. Toutefois, si l'instrument BioSorter doit être déplacé, éteignez le courant à l'interrupteur principal et débranchez le fil électrique.
- En raison du poids de l'instrument, un minimum de quatre personnes est requis pour déplacer l'instrument BioSorter. Assurez-vous que le nouvel emplacement est libre de débris et remplit les conditions de la zone de travail comme décrites dans ce manuel d'utilisation et que le chemin vers la nouvelle zone est libre d'obstruction.

4 BIOSORTER DESCRIPTION

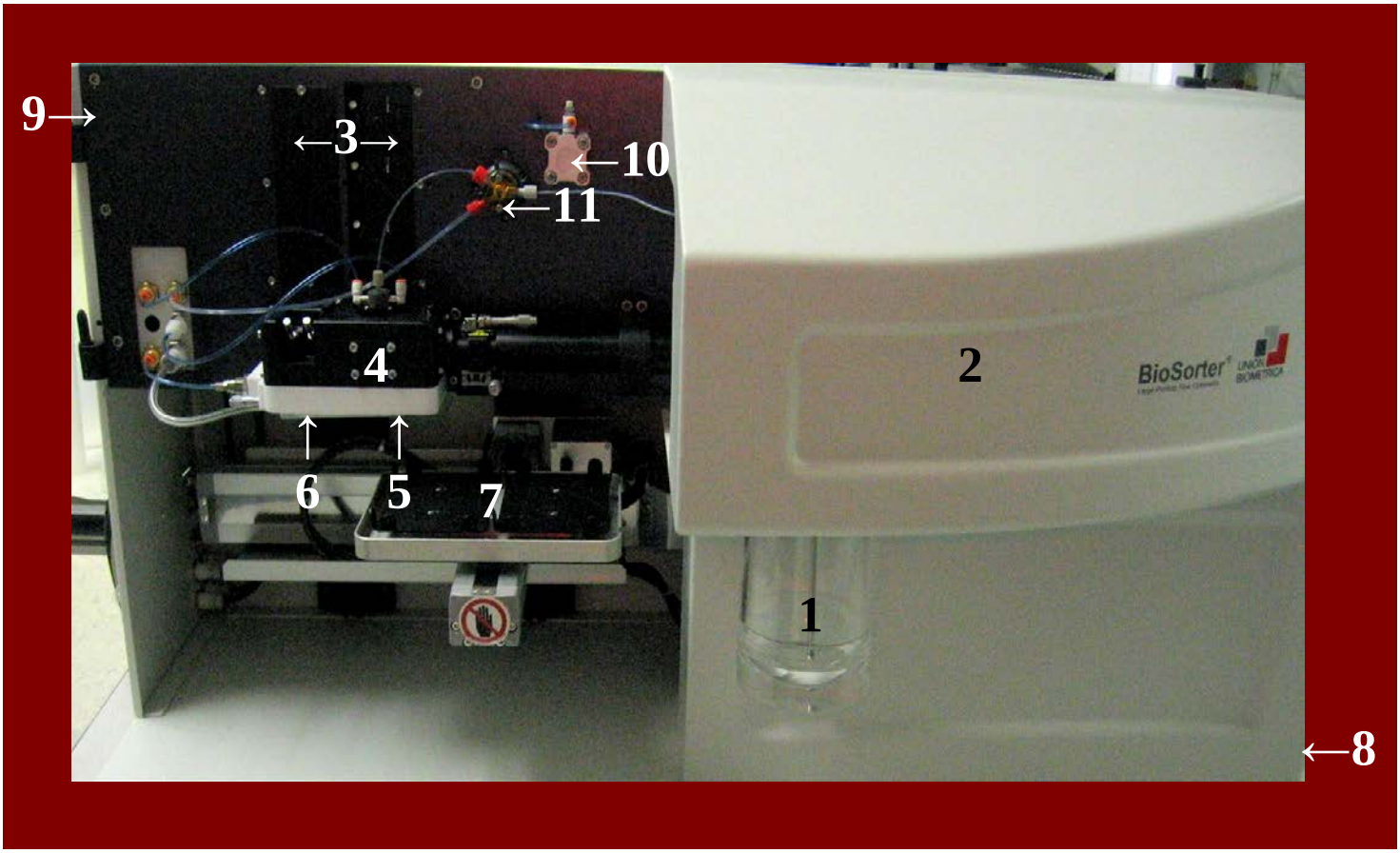


Figure 2 Operator's View of BioSorter Instrument

1. Sample Cup
2. Laser Optics Assembly
3. Flexible configuration PMT box
4. Fluidics and Optics Core Assembly (FOCA)
5. Diverter (Sorter) Valve
6. Waste Tray
7. X-Y-Z Stage
8. Power Switch
9. Sample Recovery Unit
10. Pinch Valve
11. Rotary Valve

4.1 DESCRIPTION OF COMPONENTS

4.1.1 SAMPLE CUP:

The sample cup is located to the right of the Fluidics and Optics Core Assembly (FOCA). Several options of sample cups include a 1 Liter, 1.5 Liter or disposable 50mL conical. A propeller attached to the sample probe 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.

4.1.2 LASER OPTICS ASSEMBLY

The optics assembly consists of at least one Solid state laser mounted within the optics plate. Through a series of lenses the laser beam(s) are directed to a point focused on a quartz flow cell. A photon electric sensor is located on the laser beam axis. Orthogonal emitted light is collected by PMTs (photo-multiplier tubes). The laser optics assembly should remain covered at all times.

4.1.3 FLEXIBLE CONFIGURATION PMT BOX

3 channels of fluorescence can be collected at one time using customizable PMT components. Swappable optical mirrors (dichroic mirrors) and band pass filters may be configured for specific experimental conditions.

4.1.4 FLUIDICS AND OPTICS CORE ASSEMBLY (FOCA)

The individual FOCA module is a combination of the preanalysis chamber, flow cell, and nozzle through which the sheath fluid stream is forced, producing a stabilized laminar flow. The sample is 'pushed' into the flow and focused to the center of the flow stream directly in the path of the laser(s).

4.1.5 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.

4.1.6 WASTE TRAY

A standardized waste tray is positioned directly below the nozzle. It is connected to a waste system to pump the waste stream to a waste collection container. The waste tray moves out of position to allow dispensing upon initiation of dispensing.

4.1.7 X-Y-Z STAGE

The stage supports the positioning of multiwell plates and 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.

4.1.8 POWER SWITCH

The power switch is located at the rear on the right side of the system.

4.1.9 SAMPLE RECOVERY UNIT

The sample recovery unit collects all the sample objects that are not dispensed so it can be used again. It is located behind the access panel on the left side of the instrument.

4.1.10 PINCH VALVE

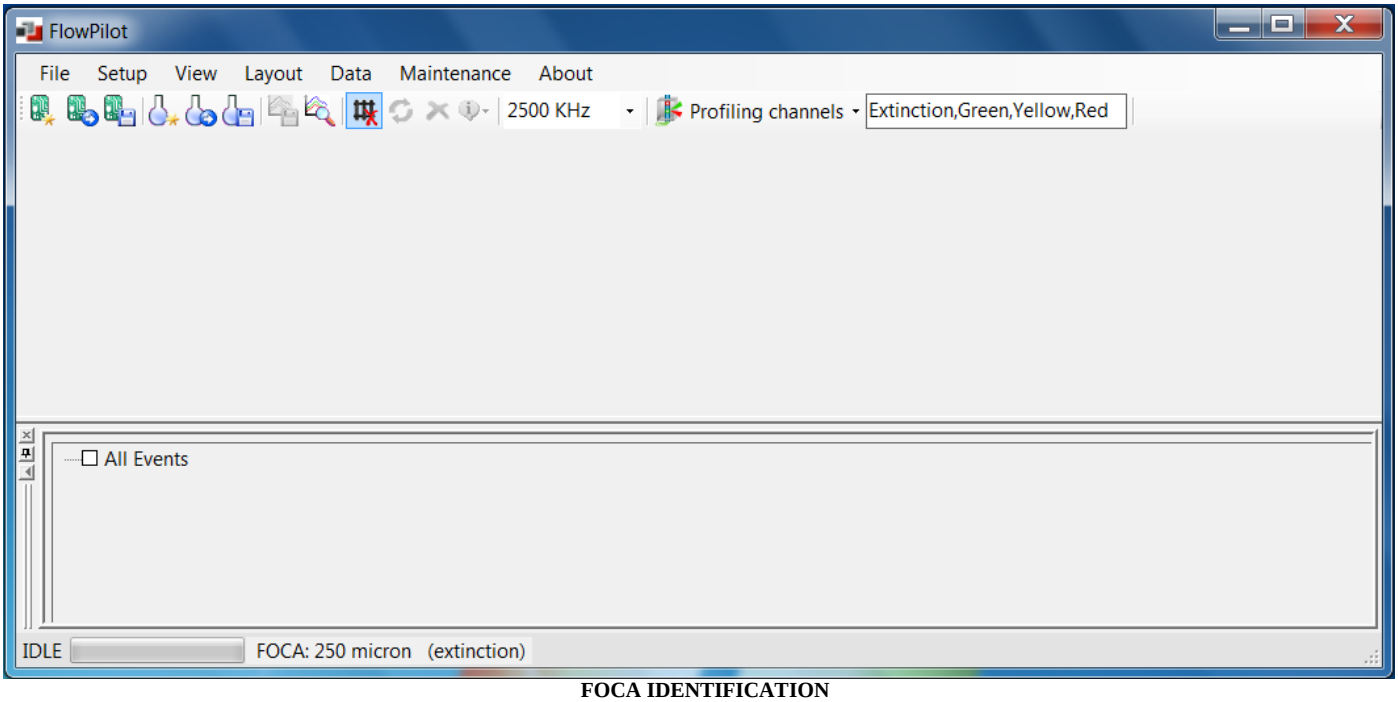
The pinch valve is used specifically for the 1000-2000 µM FOCA and utilizes 2mm silicon tubing.

4.1.11 ROTARY VALVE

The rotary valve is used specifically for the 250-500 µM FOCA and utilizes a low volume tubing.

5 OPERATING THE BIOSORTER

This chapter describes standard operation of the BioSorter instrument. Additional details and explanations of the software features can be found in Section 8 FlowPilot SOFTWARE OVERVIEW.



FOCA IDENTIFICATION

Figure 3 Screen Shot of FlowPilot-Pro Software

5.1 PREPARATION

1. Turn on the air compressor or ensure that house air is turned on and the system is pressurized.
2. Turn on the main power of the BioSorter system located on the right side of the instrument. Turn on the computer and monitor.
3. Make sure the appropriate Fluidics and Optics Core Assembly (FOCA) module is fitted correctly in the flow cell housing and that the fluid lines are attached correctly. Or swap the FOCA module according to Section 5.2CHANGING THE FLUDICS AND OPTICS CORE ASSEMBLY (FOCA) MODULE.
4. Check the waste container on the fluidics caddy (farthest left bottle on the fluidics caddy) and the sample recovery cup (inside the left side panel on the BioSorter instrument) and discard any waste contents that may have been left in the containers from previous operations.
5. Fill the sheath container (middle bottle on the fluidics caddy) and firmly tighten the cap. Refer to Section 19 REAGENTS, for information on optimal reagent usage for your application.
6. Select the FlowPilot-Pro software icon on your PC using the computer mouse. Double click the icon to open.
7. Ensure the correct FOCA module is selected for use (bottom left corner), if not go to SETUP and select CHANGE FOCA unscrew the FOCA and then toggle in place. If the software reads the correct FOCA in the bottom left corner screw the FOCA back in and select DONE.
8. Open the VIEW drop down menu and select INSTRUMENT SETTINGS to open the Instrument Settings window.
9. Open the VIEW drop down menu and select ACQUIRE/DISPENSE to open the Acquire/Dispense window.
10. Open the MAINTENANCE drop down menu and select PRIME FLOW CELL until all air has been removed from the pre-analysis chamber of the flow cell.
11. Open the MAINTENANCE drop down menu and select PRIME SAMPLE CUP until all air has been removed from sample tubing. See Section PRIME SAMPLE CUP for more information.

12. In the ACQUIRE/DISPENSE window, open the sheath valve by placing a checkmark in the box next to SHEATH ON. Place a volumetric collection receptacle (such as a graduated conical) under the flow cell, then turn off the diverter valve by un-checking the box next to DIVERTER PRESSURE and checking the box next to WASTE TRAY OPEN.
13. Measure the rate of sheath flow through the flow cell. The rates should be:

    250uM flow cell – 10mL per minute.

    500uM flow cell – 25mL per minute.

    1000uM flow cell – 45mL per minute.

    2000uM flow cell – 53-57 mL per minute.

NOTE: if sheath flow rates are not at the above conditions adjust the sheath percentage rate in the Instrument settings dialogue box. See SectionINSTRUMENT SETTINGS.

14. Turn diverter valve back on by checking the DIVERTER PRESSURE box in the ACQUIRE/DISPENSE window, turn off sheath solution by un-checking the SHEATH ON box in the ACQUIRE/DISPENSE window.
15. Run BioSorter see Section 5.4 RUNNING CONTROL PATRICLES

CAUTION: During instrument set up and sorting, the stage moves to align and accept the sorted sample into the appropriate well. Care should be taken not to place anything in the path of the stage movement. Injury and/or damage to the instrument could result.

5.2 CHANGING THE FLUDICS AND OPTICS CORE ASSEMBLY (FOCA) MODULE

NOTE: It is best to remove and store a flow cell module that is clean. Perform a cleaning step such as bleach procedure and follow with a sterile water rinse before a flow cell module is removed from the system. See section 6 MAINTENANCE of more details.

1. Make sure no fluids are moving through the system; neither sheath nor sample valves should be checked.
2. Select CHANGE FOCA from the SETUP drop down menu. A dialogue box appears instructing to change the flow cell.
3. Detach the fluid lines feeding the FOCA from the instrument panel to the left of the FOCA housing. Tubing is held in place by a pressure sleeve inside each of the red fittings. To remove the tubing, press the neck of the red fitting against the instrument and gently pull the tubing out of the fitting. The other tubing is held in place with a pressure fitting and can be removed by pressing the metal tab on top of the fitting.

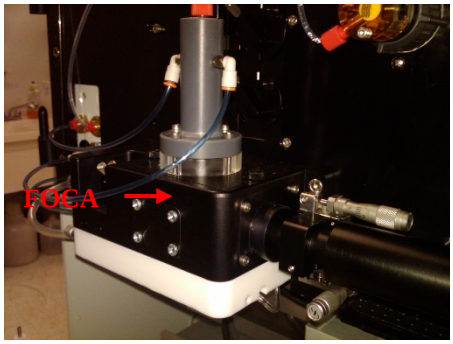


Figure 4 FOCA Housing

4. Unscrew the thumb screws holding the FOCA module into the FOCA housing. These are located just to the right and left of the FOCA as it sits in its housing apparatus.
5. Detach the sample tubing from the top of the pre-analysis chamber on the FOCA module. Allow the pre-analysis chamber to drain.
6. Lift the module from its housing and move it to a clean space for storage.
7. Place the replacement FOCA module into the FOCA housing apparatus so that the optical focusing lens (fish eye lens) is at the back of the module. Lower carefully into place so that the surface of the module is flush with the mount
8. Screw the module thumb screws into position to secure the module in place in the FOCA housing apparatus. Make sure the screws are tightly fastened.
9. Click the DONE Button on the software screen to accept the changed FOCA and enact the flow cell specific instrument settings.

10. Use an ethanol dampened kim wipe to briefly cleanse the sheath fluid line tubing (blue tubing) before placing it in the sheath line fitting (red fitting on the left). Take care to press it back into the instrument as far as it will go to ensure a tight seal.
11. In a similar manner, briefly cleanse the pre-analysis purge line tubing before placing it into its fitting on the instrument panel next to the sheath line fitting.
12. Attach the appropriate sample tubing to the top of the pre-analysis chamber and sample valve. Refer to Table 9.

NOTE: the 1000µm and 2000µm FOCA modules require use of the 2000µm silicon tubing utilizing the pinch valve.

13. At this point the instrument settings need to be set up to run the newly placed flow cell see section PREPARATION. Use control beads to verify optical alignment and instrument performance prior to running samples see section 5.1 PREPARATION. .
14. Make sure the proper waste tray is in place.
15. Align laser to the flow cell using the micrometers located just to the right of the flow cell. This needs to be done while running control particles, see section 5.4 RUNNING CONTROL PATRICLES for control particles protocol. First, locate the Extinction Detector Power reading in the INSTRUMENT SETTINGS window. By turning the bottom micrometer (with its end facing you), maximize the Extinction Detector Power reading, this should allow the user to see bead profiles in the profiler window. Turn the same micrometer until you maximize the height of the EXT peak in the profiler window. Turn the other micrometer (oriented left/right), until the EXT profile is as narrow as possible. See the optimal alignment strategy located 5.4 RUNNING CONTROL PATRICLES.

5.3 CHANGING DICHROIC MIRRORS AND FILTERS IN PMT BOX

BioSorter is customizable for specific samples/experiments. It may be necessary to change the dichroic mirrors and filters in front of the individual PMTs to collect sample specific fluorescence data.
Dichroic mirrors are optical lenses that reflect or allow transmission of emitted light to the designated PMT.
Under standard configuration the dichroic mirror in front of:
PMT 1 reflects light less than, but transmits light longer than 527nm; PMT 2 mirror reflects light less than but transmits light longer than 565nm; PMT 3 mirror reflects all light to the PMT.
Filters in front of the PMTs are also customizable.
These are bandpass filters that allow a range of emitted light to be transmitted through the filter to be collected at the PMT.
Under standard configuration:
Filter 1 (green) is a 510nm band pass filter that is 23nm wide so the range of fluorescence allowed through the filter is roughly 497 to 523nm.
Filter 2 (yellow) is a 543nm band pass filter that is 23nm wide. It collects fluorescence emission in the range of 531 to 555nm.
Filter 3 (red) is a 615nm band pass filter that is 25nm wide. It collects fluorescence emission in the range of 602 to 628nm.

5.3.1 CHANGE THE DICHROIC MIRROR.

There are several other dichroic mirrors and filters that can be swapped for sample specific staining.
To change the dichroic mirror:

1. Make sure the lasers are turned off.
2. Locate the dichroic mirror you would like to replace and remove the thumb screw that holds it in place.
3. Remove the dichroic mirror from the PMT housing, by gently pulling the fixture towards the front of the instrument until it is completely removed from its housing. Be very careful not to drop or touch the mirror with your fingers and place the mirror in a protective sleeve or box.
4. Place the new dichroic mirror in the housing pressing it flush to the instrument panel.
5. Adjust the alignment by matching the hatch marks on the mirror and the panel at the 12 o-clock position.
6. Replace the thumb screw to hold the dichroic mirror in the housing.

5.3.2 CHANGE THE BAND PASS FILTER IN FRONT OF THE PMT:

1. Make sure the lasers are turned off and locate the filters you would like to swap. Remove the filter by simply sliding it out of its housing. Immediately place it in a protective sleeve or filter box to protect the glass from scratches.

2. Identify the arrow on the edge of the filter you would like to place on the instrument. With the arrow pointing towards the left of the instrument, slide the filter into the housing in front of the correct PMT.

5.4 RUNNING CONTROL PATRICLES

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 BioSorter instrument as instructed in Section
2. PREPARATION.
3. Make sure the PMT dichroic mirrors and filter configuration is composed of the standards: green, yellow and red. Or note how the current configuration compares to the PMT configuration during past Control particle data collection. See section CHANGING DICHROIC MIRRORS AND FILTERS IN PMT BOX for more information.
4. Mix appropriate high fluorescence control particles by inversion 4 times. Avoid any excess agitation that may cause bubbles in the mixture. Refer to section 19 REAGENTS for information on optimal reagent usage for your application. Rinse sample mixer if needed before securing the sample cup.
5. Depressurize sample cup by clicking the REFILL SAMPLE button in the ACQUIRE/DISPENSE dialogue box.
6. Place a maximum of 40mL of the control particles into an approved 50mL conical tube and attach to the QuickChange sample port. If you are running beads from a larger sample cup, fill sample cup with a minimum of 100mL of control particles and replace the sample cup to the instrument.
7. Re-pressurize sample container by clicking the DONE REFILL button in the ACQUIRE/DISPENSE dialogue box. Make sure the sample cup pressure returns to the set value by looking in the INSTRUMENT SETTINGS window. It may be necessary to unscrew and refasten the sample tube or cup to its housing to ensure an adequate seal has been established.
8. Open the SETUP drop down menu and select CONTROL PARTICLES mode.

NOTE: PMTs, Threshold, Gains and Scale values are preset while in CONTROL PARTICLE mode.

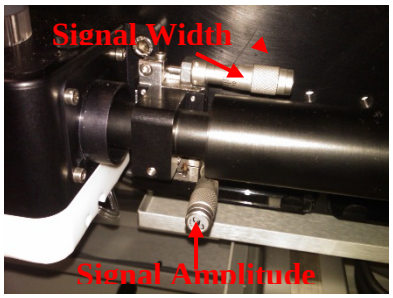


Figure 5 FOCA Micrometers

9. Click on the ACQUIRE button to begin sheath and sample flow.
10. Document the instrument's pressures found in the pressure status fields on the main screen.
11. Begin processing the beads**. Note: it will take about 1 minute for the sample to move through the tubing before it is introduced into the flow cell.** Adjust the sample pressure (found in the sample pressure field in the INSTRUMENT SETTINGS dialogue box) in small increments to increase or decrease the sample pressure in order to obtain an acquisition rate of 5-10 beads per second. Once adequate pressure has been established erase the data by double clicking on the red "X" under the main menu items. Then collect a minimum of 500 events. At least 500 events are required to obtain statistical data. The signal amplitude micrometer moves the lens adjusting the focal point inside the flow cell to maximize the extinguish amplitude. The signal width micrometer focuses the laser to minimize the signal width. By achieving the maximum amplitude and narrowest width signal the system will perform at an optimal level.

Optimal strategy: Move the amplitude micrometer to the right or left while monitoring the power reading at the extinction detector. As the power reading rises the laser is being brought into focus at the center of the flow cell—beads should become visible by the instrument. Once beads are being detected, reduce the sample pressure slightly in order to narrow the sample stream. The bead signals should be visible on the average value focusing plots (at the right of software screen) in the control particle Flow Pilot window. The top left window displays the extinction peak amplitude, the lower left window displays the extinction width. The three displays on the right correspond to the green, yellow, and red fluorescence amplitudes. Continue to make small changes to the amplitude micrometer until the graphical line tracing the average extinction signal intensity can no longer be increased by changing the position of the amplitude micrometer. Next

make small changes to the width micrometer while monitoring the average width trace. At some coordinate combination, the extinction amplitude and widths as well as the fluorescence will be optimized.

12. Document the MEAN and C.V. of the particles from the top histogram titled TOF. 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 laser is operating properly for obtaining TOF and EXT readings and also that the optics are clean and in working order.

NOTE: Shifts in the Mean or C.V. of the particles may indicate systematic problems with the Dispenser. (See Sections 8 **FlowPilot SOFTWARE OVERVIEW, and Section 21 TROUBLESHOOTING)**

5.5 FLUSHING PROCEDURE

NOTE: If the sheath and the sample valves are left open while the sample cup is depressurized, the sheath fluid will flush back into the primary sample cup. This can be a useful method for removing clogs or objects from the sample tubing, but will result in contamination of your sample.

1. Press the UNCLOG button on the ACQUIRE/DISPENSE dialogue box once. This initiates a clean cycle sending the contents of the sample line back into the sample cup.
2. Turn off sample valve by un-checking the SAMPLE ON box in the ACQUIRE/DISPENSE dialogue box.
3. Depressurize sample container by clicking the REFILL SAMPLE button in the ACQUIRE/DISPENSE dialogue box.
4. Remove sample cup from sample port, UNCLOG mixer if needed, and replace with a container filled with distilled water.
5. Re-pressurize sample container by clicking the DONE REFILL button in the ACQUIRE/DISPENSE dialogue box. Make sure the sample cup pressure returns to the set value by monitoring the sample pressure field in the INSTRUMENT SETTINGS dialogue box.
6. Select the SAMPLE ON check box in the ACQUIRE/DISPENSE dialogue box. A warning message will appear, click OK and process the water until the sample cup is nearly empty. If sample is not to be run immediately be sure to close the sample valve when a minimal amount of water remains in the sample cup.
7. Once flushed with 20 mL distilled water, uncheck SAMPLE ON so not to run the sample dry

5.6 RUNNING SAMPLES

NOTE: If a sterile sort is required, perform Sterilization Procedure according to section COMPLETE STERILIZATION PROCEDURE.

5.6.1 ANALYSIS

1. Remove objects from sample tubing by using the flushing procedure outlined in Section 5.5 FLUSHING PROCEDURE.
2. Depressurize sample container by clicking the REFILL SAMPLE button in the ACQUIRE/DISPENSE dialogue box.
3. Fill approved 50mL conical tube with a minimum of 5mL and a maximum of 40mL of sample and attach to the QuickChange sample port.
4. Re-pressurize sample container by clicking the DONE REFILL button in the ACQUIRE/DISPENSE dialogue box. Make sure the sample cup pressure returns to the set value by looking in the INSTRUMENT SETTINGS dialogue box.
5. If in CONTROL PARTICLE mode, open the SETUP drop down menu and exit from CONTROL PARTICLE mode by removing the checkmark next to this feature.
6. Open an existing experiment or define new conditions for running the sample.
7. To open an existing experiment and sample:

a. In the FILE menu choose OPEN EXPERIMENT. Choose the directory and experiment file to open. Click OK to initiate the opening of the experiment. When this is opened all the instrument settings used during that experiment will be imported and used as the current configuration settings.

b. Open an existing sample file. In the FILE menu, choose OPEN SAMPLE. Choose the directory and appropriate sample file to open. Click OK. When this is opened all histograms, plots, regions and other sample specific settings will be active for new sample acquisition.

8. To define new conditions for running your sample:

   a. Open the VIEW menu and select INSTRUMENT SETTINGS.

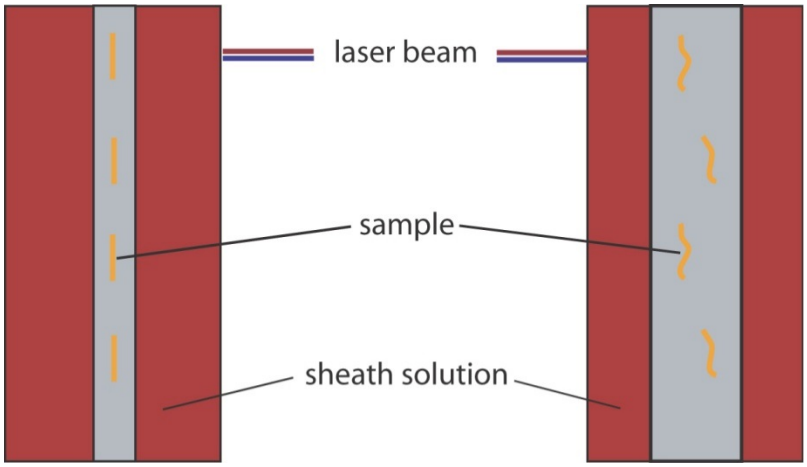
   b. Set all the parameters for acquiring your samples, including which lasers, laser powers, gains, etc for your data collection.

   c. Open the LAYOUT drop down menu and open as many data windows as necessary. It is useful to open at least one histogram, density plot, and profiling window as a default. See section 12 LAYOUT MENU FEATURES for more information regarding available data windows.

9. Click the ACQUIRE button in the ACQUIRE/DISPENSE dialogue box to begin sheath and sample flow. The system will prompt you to enter a file name to store the data about to be acquired. Input a file name and Click OK to initiate sample acquisition. This will save the data in several file formats that can be reviewed later using the FlowPilot software.

10. System takes a moment to open the sheath and sample valves and will take about 1 minute for the sample objects to travel to the flow cell.

11. Monitor sample acquisition and the sample moving through the sample tubing. Adjust the sample concentration, if necessary, in order to obtain stable flow of the objects (10-30 events per second).



12. Pause the data collection by pressing the PAUSE button in the ACQUIRE/DISPENSE dialogue box. And close the sample valve by removing the check box next to Sample on in the ACQUIRE/DISPENSE dialogue box. Select a stored file for sample processing or adjust the instrument parameters for proper analysis.

Figure 6 Correct Object Alignment

13. Make adjustments to instrument settings or plots as necessary to better identify the various characteristics of the sample. Left click on a displayed axis to choose from a list of other parameters to view. Right clicking within the body of a graph will allow user to modify the scales, apply gates, draw and modify regions. More detailed instructions for use can be found in the appropriate sections in Section 12.2.1 MODIFY THE DOT/DENSITY PLOTS.

14. Analyzed objects will be retained in the sample recovery container. Contents can be transferred to another receptacle for storage or reuse.

5.7 SORTING (DISPENSING THE SAMPLE)

For further SORT details and instructions, refer to sections: 8.3 DATA REPRESENTATION, 11 VIEW MENU FEATURES, and 12 LAYOUT MENU FEATURES.

5.7.1 DISPENSING OBJECTS

The instrument checks to see that an object fulfills all sort criteria according to the conditions set for dispensing in a drop directly below the flow cell nozzle.

1. Define sort criteria as described in ANALYSIS and VIEW sections of the manual.
2. Select which region you'd like to dispense. Under the SORTING heading in the ACQURE/DISPENSE dialogue box, choose the sorting gate from the drop-down list of all active regions contained in your sample file.
3. For bulk sorting, input the number of objects from the defined region to dispense to a collection device directly below the flow cell nozzle. This field is located right under the SORTING GATE heading in the ACQUIRE/DISPENSE dialogue box. Then proceed to step 7.
4. For Dispensing to a multi-titer plate, select the name of a calibrated plate with the correct well configuration from the PLATES heading under the SETUP menu. If using a new plate, the plate positions need to be calibrated before dispensing. Follow the steps outlined in the PLATES heading found in the SETUP menu feature 10.3 SETUP MENU FEATURE.
5. Open the plate template, by selecting PLATE TEMPLATE from the VIEW menu.
6. Input each location, the fill order and the number to dispense across the plate. Click OK to implement the template.
7. After sorting number and locations have been assigned, click BULK SORT or FILL PLATE buttons from the ACQUIRE/DISPENSE dialogue box to initiate dispensing in bulk or to a multi-titer plate.
8. Verify sorting accuracy by looking at dispensed objects under a microscope. Manually make changes to delay and width settings or select Sort Delay setup in the instrument settings dialogue box and follow prompts to test delays to obtain the best delay settings for the flow cell being used and your sample. See Section 11.1 INSTRUMENT SETTINGS.

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. Stacking two multiwell plates in the X-Y-Z stage decreases the distance between the nozzle and the multiwell plate allowing the droplet to retain its form.

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.

5.8 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.

5.8.1 ADDING TO THE SAMPLE CUP

1. Close the SAMPLE valve by unchecking the SAMPLE valve checkbox (Acquisition is not stopped).

2. Unscrew and remove the sample cup from the instrument.
3. Refill and replace the sample cup on the instrument.
4. Allow 30 seconds for sample cup to repressurize.
5. Restart SAMPLE valve by re-checking the checkbox.
6. Continue sorting.

5.9 ADDING SHEATH REAGENT DURING OPERATION

CAUTION: The *BioSorter* is a pressurized system.

1. Close the sample and sheath valves by unchecking the SAMPLE and the SHEATH checkboxes.
2. Open the cap of the sheath container and fill as needed. Replace the cap and firmly tighten.
3. Open the SHEATH valve to reestablish sheath flow.

5.10 UNCLOGING

If flow rate decreases or any other flow problems occur, click the UNCLOG button to clear the sample line prior to attempting any troubleshooting of the instrument. See section 21 TROUBLESHOOTING for other options.

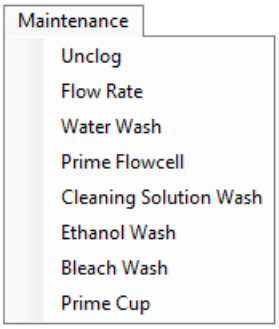
Whenever a biological sample has been run through instrument it is necessary to perform a system cleanse with any one or combination of cleansing reagent protocols: bleach procedure, ethanol sterilization, or cleaning solution, followed by thorough rinsing with deionized/distilled water. See Section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

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

5.11 INSTRUMENT SHUT DOWN

1. After system has been properly cleaned, see Section 6 MAINTENANCE, double click the X at the top right corner of the software screen.
2. A dialogue box will appear asking if the user would like to save the experiment and sample settings that were used. Yes will allow the user to store the experiment and samples under new names. No will not store any of the settings used during this data acquisition before closing the software.
3. Turn off the instrument using the switch on its right side.
4. Shut down the computer and monitor.
5. Switch OFF the air-compressor.

6 MAINTENANCE



enance Menu

The following contains the Daily Maintenance procedure and should be performed by the Operator at least once daily when the instrument is in use.

Daily, short/long term shutdown, and sterilization maintenance worksheets are found in section APPENDIX B: Log Sheet 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.

Recommended maintenance for other parts are given in this section as well. The schedule is based upon average use of the BioSorter instrument.

6.1 DAILY MAINTENANCE PROCEDURE

1. Place the appropriate solutions in the containers on the Fluid Caddy see section 7 EXTERNAL FLUIDS CADDY for information on the appropriate locations for each specific solution and section 19 REAGENTS for concentrations.
2. Tightly secure an empty 50 mL conical on the instrument.
3. Go to MAINTENANCE and select PRIME SAMPLE CUP.
4. Go to VIEW and select ACQUIRE AND DISPENSE in this menu select to open the sample valve.
5. Go to MAINTENANCE and select BLEACH WASH this process should take approximately eight minutes.
6. Once bleach wash is completed go to MAINTENANCE and select CLEANING SOLUTION WASH again this process will take eight minutes.
7. Once cleaning solution wash is completed remove the conical and rinse the remaining solution out.
8. Tightly secure the conical back to the instrument.
9. Go to MAINTENANCE and select WATER WASH this process will take five minutes.
10. Repeat WATER WASH two more times.
11. Remove conical and rinse the remaining solution out before proceeding with running a sample.
12. Open and empty the sample recovery cup and remove the cell strainer from its housing. Wipe away any debris or growth before remounting cell strainer and sample recovery cup.

NOTE: The BLEACH, CLEANING SOLUTION, or ETHANOL WASH can be used during steps 4 and 5, but be sure to repeat WATER WASH before proceeding with running a sample.

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

6.2 RECOMMENDED MAINTENANCE SCHEDULE

Union Biometrica recommends that a Preventative Maintenance (PM) service call be performed at least once annually for continued high performance of your instrument. During the PM Visit, a Union Biometrica service engineer changes all fluidic tubing, gaskets and fittings; cleans and adjusts the system optics, FOCA and diverter valve; tests the overall laser life; and fully calibrates the system for optimum performance. In addition to the yearly PM, the following maintenance schedule is recommended. Actual maintenance needs may vary based upon instrument usage.

Item	monthly	yearly	every 2 years	other
Preventive Maintenance (PM) Visit		schedule		
Peristaltic Pump Tubing (2x)	check	replace		
Sample & Prime Tubing	check	replace		
Sample Cup O-Ring(s)	check	replace		
Stirring Probe belt		replace		
Waste Tray	clean	check / replace		
Fluid Tubing		check / replace		
Fluid Line Sterility Filters	check	replace		
Air Line Sterility Filter		check	replace	
Sheath Pump Heads		check	replace	
Diverter Valve		check	replace	
Optical Filters		check		over time filters become less effective at blocking light
Lasers		check		replace every 10,000-20,000 hours depending upon laser

Table 1 Recommended Maintenance Schedule

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.

Complete Bleach Sterilization.

1. Make sure there is a flow cell module in place on the instrument and that the fluid lines are all correctly connected.
2. Close the SAMPLE and SHEATH VALVES by removing checks from their checkboxes.
3. Detach and remove sterility filters from the Sheath and Water lines on the right side of the External fluids caddy. Replace the removed filters with two patch tubing lines supplied with the instrument.
4. Remove the cap from the Sheath and Water containers, and empty their contents.
5. Rinse each container with deionized/distilled water.
6. Fill the Sheath and Water container with 1 liter of freshly made 50% Bleach Solution (final hypochlorite concentration should be ~2.5%) see section 19 REAGENTS for more details.
7. Carefully swirl the bleach in the containers to sterilize all the interior surfaces.
8. Replace and firmly tighten the cap to the sheath container.
9. 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.
10. Monitor the waste output to ensure the bleach solution is flowing through the system.
11. Close the SHEATH VALVE by unclicking the sheath valve checkbox.
12. Go to MAINTENANCE and select WATER WASH.
13. Thoroughly rinse the bleach from the sheath and water container and replace with 1 liter of distilled water.
14. Open the SHEATH VALVE by clicking the sheath valve checkbox. Allow a few minutes for the water to flood the Sheath line and Flow Cell.
15. Ensure the appropriate solutions are in the cleaning containers see section 7 EXTERNAL FLUIDS CADDY.
16. Go to MAINTENANCE and select BLEACH WASH.

17. Once completed go to MAINTENANCE and select WATER WASH.
18. Repeat WATER WASH two more times.

Complete Ethanol Sterilization.

19. Ensure SAMPLE and SHEATH VALVES are closed (check marks are removed from checkboxes).
20. If sterility filters are present, detach and remove the sterility filters from the Sheath and Water lines on the right side of the external fluids caddy. Replace the sterility filters with the 2 patch tubing supplied with the instrument.
21. Remove the caps from the Sheath and Water containers, and empty their contents.
22. If necessary, rinse each container with deionized/distilled water.
23. Fill both the Sheath and Water containers with 1 liter of 70% Ethanol see section 19 REAGENTS for more details.
24. Carefully swirl the Ethanol in each container to sterilize all the interior surfaces.
25. Replace the caps and attach the fluid lines to both the Sheath and Water containers.
26. Open the SHEATH VALVE by clicking the sheath valve checkbox. Allow a few minutes for the Ethanol to flood the Sheath lines and Flow Cell.
27. Monitor the waste output to ensure the ethanol solution is flowing through the system.
28. Go to MAINTENANCE and select WATER WASH.
29. Close the SHEATH VALVE by unclicking the sheath valve checkbox.
30. Thoroughly rinse the ethanol from the sheath and water container and replace with 1 liter of distilled water.
31. Open the SHEATH VALVE by clicking the sheath valve checkbox. Allow a few minutes for the water to flood the Sheath line and Flow Cell.
32. Ensure the appropriate solutions are in the cleaning containers see section 7 EXTERNAL FLUIDS CADDY.
33. Go to MAINTENANCE and select ETHANOL WASH.
34. Once completed go to MAINTENANCE and select WATER WASH.
35. Repeat WATER WASH two more times.

Apply sterility Filters to the SHEATH and WATER lines using sterile technique.

36. Press the ABORT button once (in the ACQUIRE/DISPENSE dialogue box) to stop all fluids from moving through the system.
37. 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 FLOW RATE if necessary to establish sheath fluid movement.
38. Press ABORT button once to stop all fluids from moving through the system.
39. Sterilize the area on the External Fluids Caddy where the sterility filters will be applied. Spray all the surfaces around the connections with 70% ethanol.
40. 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).
41. Remove the filter by releasing the quick connectors and install a new filter.
42. Spray both the connectors on the External Fluids Caddy and the ends of the sterility filter with ethanol before fitting them together.
43. Repeat these steps to apply the sterility filter to the Water container.

Rinse all lines with sterile deionized/distilled water.

44. 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 FLOW RATE if necessary to establish sheath fluid movement.

45. If necessary debubble the sheath sterility filter and fluid lines: Tapping the sterility filter sometimes dislodges some bubbles. 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.
46. Press the UNCLOG button a few times to allow the sterile water to wet the filter and rinse the sample line.
47. Initiate a WATER WASH to debubble the Water filter in a similar manner.

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.

48. Open the SHEATH VALVE to allow the water to continue to rinse the sheath lines for 10-30 minutes. UNCLOG button may be pressed several more times during this time period but do not allow the containers to fully empty.

NOTE: If using 1or 2 liter sample cup rinse with sterile water. Used 50 mL conicals can be disposed and replaced with sterile unused ones.

49. Click OFF the SAMPLE and SHEATH VALVE checkboxes.
50. Remove the sample cup/conical and remove any ethanol left in the sample cup.
51. Manually rinse the cup with bleach and ethanol.
52. Rinse the cup/conical with sterile deionized/distilled water several times.
53. Fill the cup with sterile water and replace it on the instrument.
54. 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.
55. Process the water until the sample cup is nearly empty.
56. Click the ABORT button once to stop all fluids moving through the system.

Establish appropriate Sheath flow.

57. While no fluids are moving through the system, carefully open and empty the Sheath and Water containers of the sterile water.
58. Refill each container with the appropriate sheath fluid and replace the container caps.
59. Open the SHEATH VALVE and allow sheath to flood the tubing and allow it to process for several minutes before closing the SHEATH VALVE.
60. Check the sheath flow rate by turning the DIVERTER PRESSURE OFF and WASTE TRAY ON.
61. Measure the flow rate for one minute and determine if appropriate for the given FOCA. Adjust the sheath flow rate as needed.
62. 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.

63. Ensure no fluids are moving through the system. Carefully remove the waste tubing from the base of the waste tray.
64. Click the waste tray open check box and unscrew the set pin at the back of the waste tray to move the waste tray out from under the nozzle. Carefully slide the waste tray from its holder to completely remove it from the instrument.
65. Disconnect the waste tubing from the instrument..
66. Using 70% ethanol dampened KIM wipes and/or cotton applicators (Q-tips), sterilize all the surfaces of the waste tray holder: sliders, diverter, and the aperture below the flow cell nozzle.
67. Thoroughly spray the waste tray with 70% ethanol to sterilize all its surfaces before putting it back in position.
68. 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.
69. Allow the tubing to air dry or rinse it in a similar manner with sterile water.
70. Ethanol cleanse the end of the tubing before refitting it to its housing on the instrument body.

71. Open and empty the sample recovery cup and remove the cell strainer from its housing.
72. 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.
73. Remove and empty the recovery cup and rinse with sterile water.
74. 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.
75. 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.
76. 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 BioSorter instrument.

6.4 REPLACING THE WASTE PUMP TUBING

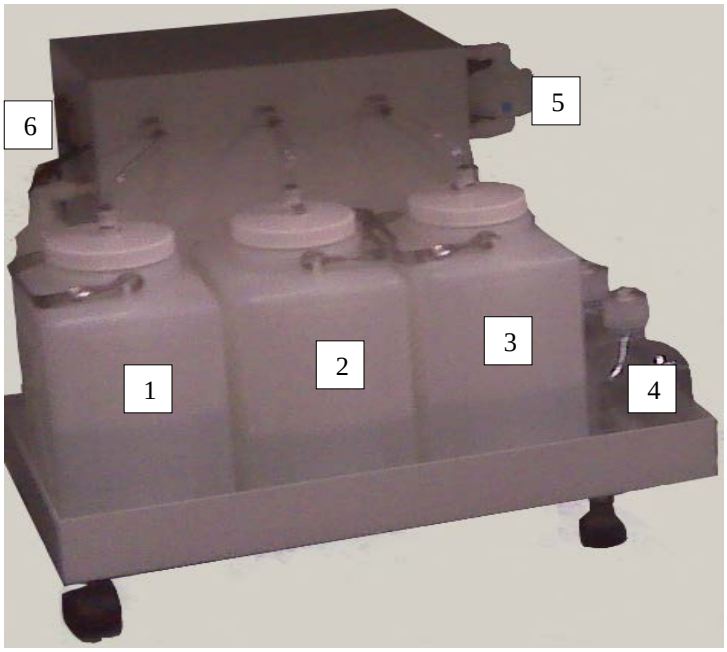
This procedure is periodic, depending on usage. Visual inspection of the waste pump tubing is recommended on a regular basis. The appearance of dry, cracked tubing or pooling under the waste tray is an indication that the waste pump tubing needs to be replaced. Follow the steps below to change the tubing.

1. Click the ABORT button twice in the BioSorter software if the instrument is ON.
2. Remove waste pump tubing by lifting the clamp pressing the tubing over the waste pump. Then detach both connectors from their fittings.
3. Using the old tubing as a guide cut a new length of tubing and attach to the connectors.
4. Install the new tubing by laying it over the waste pump rollers and tightly closing the clamp over the tubing.
5. Open the sheath valve to verify that fluid is pumping through the waste pump tubing to the waste bottle and, if not, readjust the placement of the waste tubing within the pump/clamp housing.

6.5 REPLACING THE PRIME TUBING

1. If an error occurs during priming the system, the priming tubing may need to be replaced.
2. Remove priming tubing from the box located in front of the waste pump housing.
3. Using the old tubing as a guide, cut a new length of tubing.
4. Install the new tubing.
5. Go to MAINTENANCE and select PRIME FLOW CELL to verify the tubing is working properly.

7 EXTERNAL FLUIDS CADDY



1. Waste Container,
2. Sheath Container
3. Water Container
4. System Cleaning Containers
5. Fluid Line Sterility Filter
6. Pressure Regulator

Figure 8 Operator's View of BioSorter Fluid Caddy

7.1 DESCRIPTION OF COMPONENTS

7.1.1 WASTE CONTAINER:

A 9 liter container where all fluids moving through the instrument are accumulated. Instrument has fluid level sensor and will flash a warning message indicating waste should be emptied before the bottle overflows.

7.1.2 SHEATH CONTAINER:

A 9 liter bottle can be filled with appropriate system sheath such as cell sheath reagent. Container is equipped with a fluid level sensor and will flash a warning message when fluid in the sheath container gets low reminding the user to refill the sheath container.

7.1.3 WATER CONTAINER:

A 9 liter bottle filled with water for cleaning/rinsing cycles during instrument use. Container is equipped with a fluid level sensor and will flash a warning message when the fluid level is low and needs to be refilled.

7.1.4 SYSTEM CLEANING CONTAINERS:

1 liter bottles containing cleaning solutions. The container next to the water bottle contains a 50% bleach solution. Next are a 70 % ethanol solution and finally a container of cleaning solution see section 19REAGENTS for more details. These procedures are located in the MAINTENANCE feature.

7.1.5 FLUID LINE STERILITY FILTERS:

0.22um sterility filters are in place to filter the Sheath and Water fluid lines entering the instrument.

7.1.6 PRESSURE REGULATOR:

Pressure is supplied by an external compressor (or house air) and is regulated for the system at approximately 40 PSI. The pressure regulator allows adjustment and monitoring of the pressure.

8 FlowPilot SOFTWARE OVERVIEW

8.1 START UP

The following picture shows the main screen of the BioSorter 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.

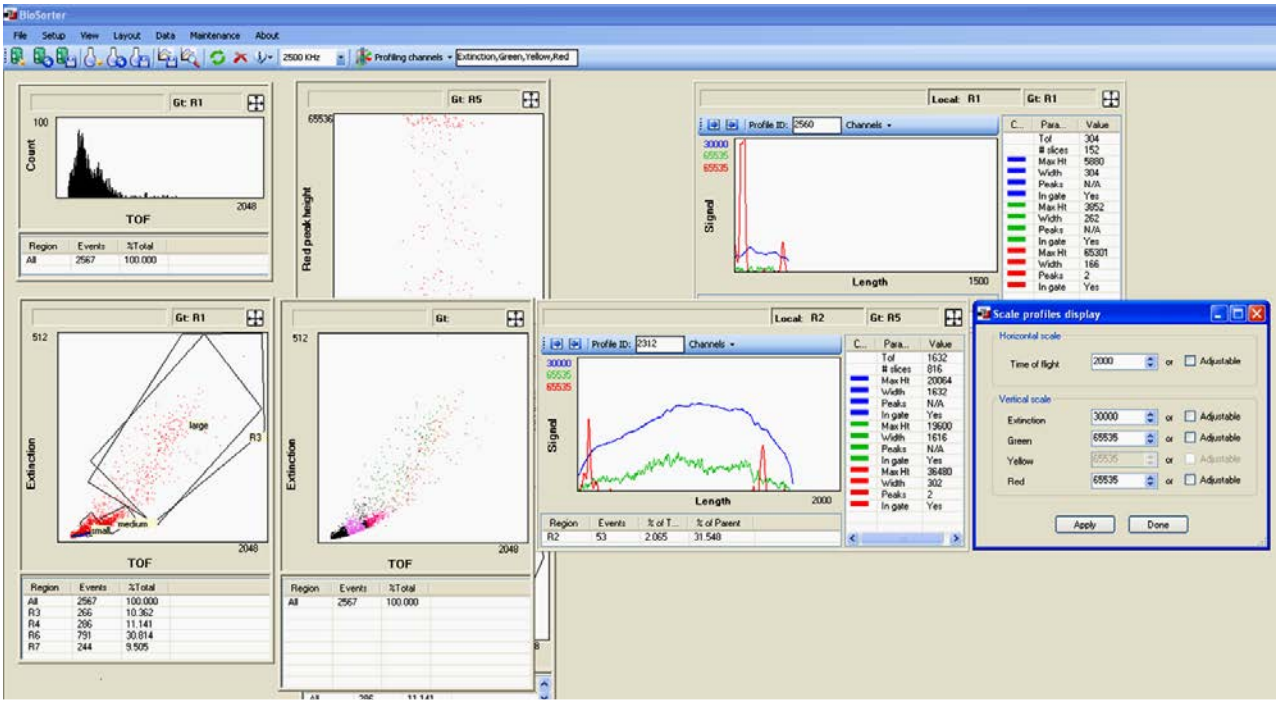


Figure 9 FlowPilot-Pro Software with Plots

8.2 ICONS EXPERIMENT AND SAMPLE CONTROLS

8.2.1 NEW OPEN/SAVE/SAVE AS EXPERIMENT

New Experiment

Click the NEW EXPERIMENT icon to define a new experiment.

NOTE: all instrument settings will need to be redefined once a new experiment is established.

Open Existing Experiment

Click the OPEN EXPERIMENT icon to import the instrument settings from a previous experiment to be used currently on the instrument.

Save Experiment

Click the SAVE EXPERIMENT to save the current settings to a new experiment file.

NOTE: it is best to define a new experiment without overwriting one previously defined.

8.2.2 OPEN/SAVE/SAVE AS SAMPLE

New Sample

Click the NEW SAMPLE icon to define new sample/conditions for data acquisition within the current experiment.

Open Sample

Click the OPEN SAMPLE icon to open and existing sample template.

Save Sample

Click the SAVE SAMPLE icon to save the current sample template as a new sample file.

NOTE: it is best to define a new sample instead of overwriting one previously defined.

8.2.3 OTHER ICONS

Erase Data

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

Refresh Data

Click REFRESH icon to refresh the data and statistics displayed.

Store Data

Click the STORE icon to store the current data acquisition.

Review Data

Click the REVIEW icon to view previously acquired data.

8.3 DATA REPRESENTATION

8.3.1 SCAN RATE

The current instrument scan rate is displayed on the icon bar of the software screen. To change the scan rate or for more information about determining the best scan rate, see scan rate section in 10.6.1 Scan rate.

8.3.2 PROFILING CHANNELS

Display of the profiling channels currently set for data collection: Extinction, PMT 1 (Green), PMT2 (Yellow), PMT3 (Red)

To change the profiling channels, left click on the profiling heading and select/deselect individual profiling channels.

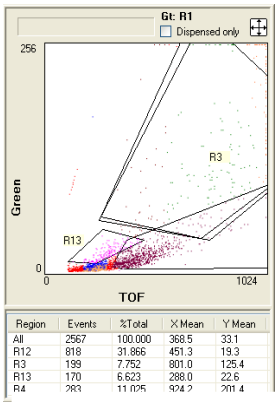


Figure 10 Dot Plot

8.3.3 HISTOGRAM, DOT PLOT, AND PROFILING GRAPH DISPLAY

Multiple histograms, dot plots and profiling graphs can be displayed on the software screen during data collection and analysis. To use see Section 12.2 ADD DOT PLOT and ADD DENSITY PLOT

Statistics display

At the bottom of each histogram and dot plot is a summary of statistics calculated for data contained on that histogram or plot. See section 12.8 STATISTICS for more information.

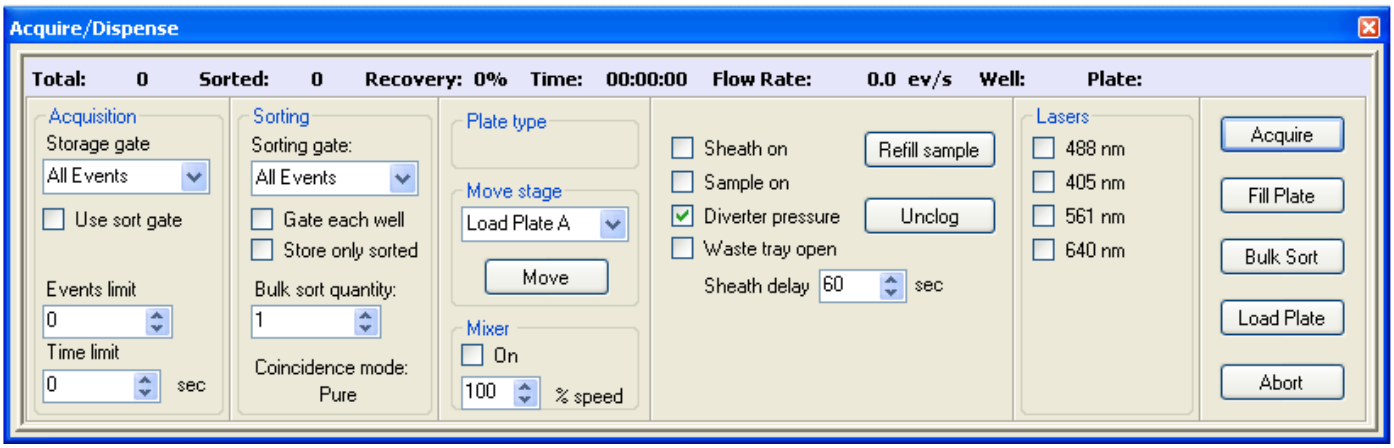


Figure 11 Acquire/ Dispense Menu

8.4 DOCKED INSTRUMENT SETTINGS, ACQUIRE/DISPENSE, AND GATING HIERARCHY DISPLAY

It is possible to dock menu windows by dragging and dropping the menu display of the instrument settings, acquisition/dispense and gating hierarchy to the Flow Pilot screen.

Left Click and hold on the active menu window and drag to the right, left, or bottom of the Flow Pilot tablet. A highlighted box will appear indicating the location where the menu will be dropped. Once desired location is highlighted, release the menu to drop it into position. Additional menus can be added as tabs or in series to the new tool bar in the same manner where the highlighted area indicates the new location of the dropped menu. The menus can be pinned (using the push pin Icon) to this new location. To move a docked menu, grab the left edge of the menu window and drag to a new location. These menu features are found in the View menu heading and are discussed in section 11 VIEW MENU FEATURES.

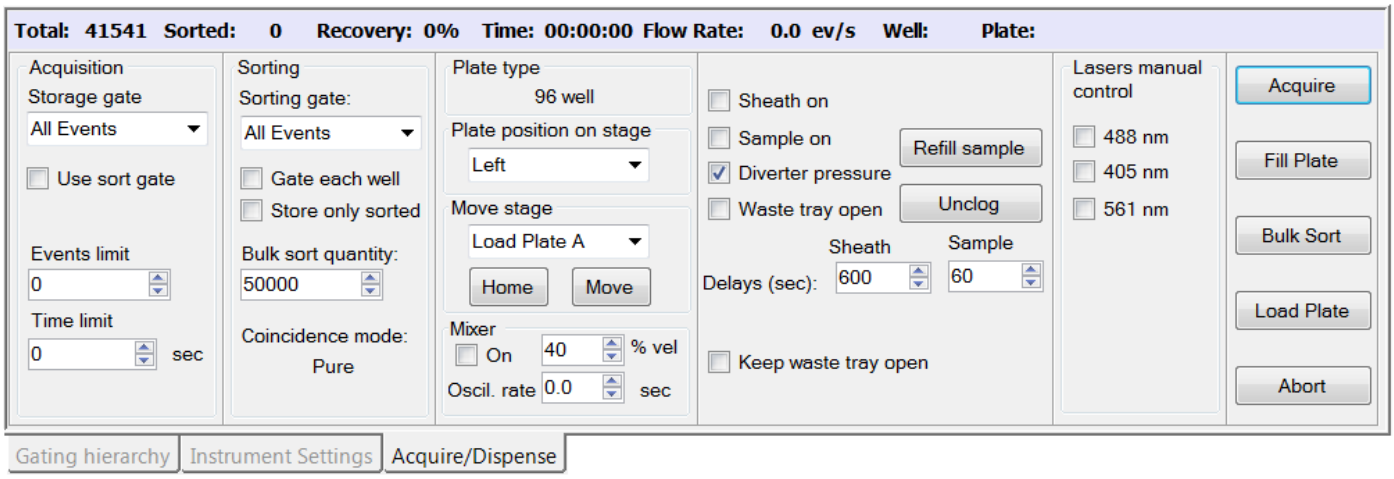
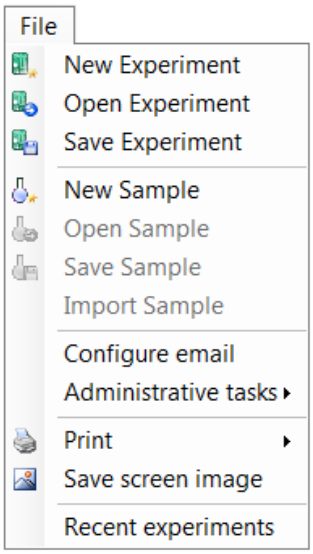


Figure 12 Docked Settings, Acquisition and Gating Hierarchy

9 FILE MENU FEATURES

9.1 NEW EXPERIMENT

Click NEW EXPERIMENT to define a new experiment. Note: all instrument settings will need to be redefined once a new experiment is established. Upon selecting this feature, system will ask user if they would like to save the current experiment. Click yes to save the current settings under a new name or NO to start with a fresh FlowPilot screen. Experiment settings (those in the instrument Settings dialogue box will need to be defined for the new experiment.



Menu

This feature can also be performed by clicking on the NEW EXPERIMENT icon on the tool bar.

9.2 OPEN EXPERIMENT

Click OPEN EXPERIMENT to import the instrument settings from a previous experiment to be used currently on the instrument. Upon selecting this feature, system will ask the user if they would like to save the current Experiment settings. Choose Yes to save the current settings under a new experiment file name.

This function can also be performed by clicking the OPEN EXPERIMENT Icon from the tool bar.

9.3 SAVE EXPERIMENT

Click SAVE EXPERIMENT to save the current settings to a new experiment file. Note: it is best to define a new experiment without overwriting one previously defined.

This function can also be performed by clicking on the SAVE EXPERIMENT Icon on the tool bar.

9.4 NEW SAMPLE

Click NEW SAMPLE to define new sample/conditions for data acquisition within the current experiment. Sample settings include: all histograms, plots regions, gates and profiling criteria.
Upon selecting this feature, the system will ask user if they would like to save the current sample. Click yes to save the current settings with a new sample name or no to erase the sample and start with a fresh FlowPilot screen. Define new histograms, plots and graphs as desired.

This function can also be performed by clicking on the NEW SAMPLE Icon on the tool bar.

9.5 OPEN SAMPLE

Click OPEN SAMPLE to open an existing sample. Upon selecting this feature, system will import all the sample conditions (histograms, plots, regions, gates, profiling criteria) stored in that sample file.

This function can also be performed by clicking the OPEN SAMPLE Icon on the tool bar.

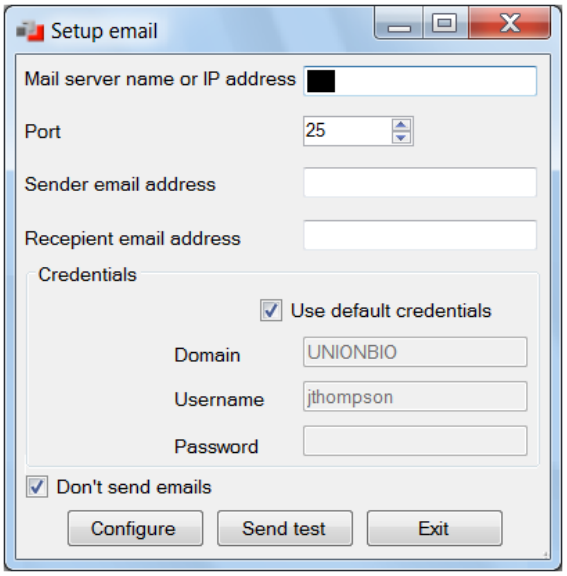
9.6 SAVE SAMPLE

Click SAVE SAMPLE to save the current sample conditions. Input a name to save the new file within the define experiment folder. Note: it is best to save the sample as a new name rather than overwrite an existing sample file. This feature can also be performed by clicking the SAVE SAMPLE icon on the tool bar.

9.7 IMPORT SAMPLE

Click IMPORT SAMPLE to import an existing sample saved under a different experiments directory. Upon selecting this feature, system will import the entire sample conditions (histograms, plots, regions, gates, profiling criteria) stored in that sample file.

9.8 CONFIGURE EMAIL



User may input addresses and accounts to setup email notifications. User must input:

Mail server name or IP address

Port

Sender email address

Recipient email address

Credentials including domain, username, and password

9.9 ADMINISTRATIVE TASKS

Activating this feature, allows the user to select specific files and launch several administrative tasks including:

Update configuration

Update maintenance

User may choose to update individual or all procedures

Backup all

Runs a procedure to backup all FlowPilot specific files.

Restore from backup

Allows system to reinstall a backup file configuration.

9.10 PRINT

Use this shortcut button to open the printing dialogue box.

9.11 SAVE SCREEN IMAGE

Click Save screen image to save a screen image of the current software screen. User can choose to save the image of the entire software screen subsets with various file format options, including:

Captured area

Choosing either the entire screen or the layout only.

File Format

Select either Bitmap, JPEG, Tif file, or Portable Network Graphics file

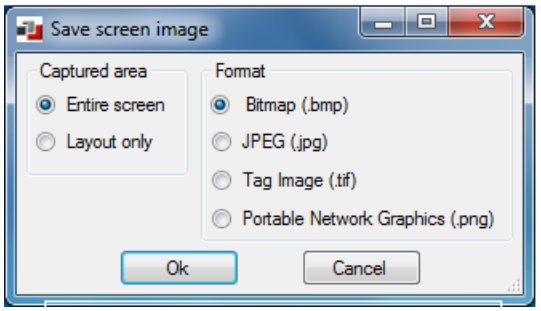
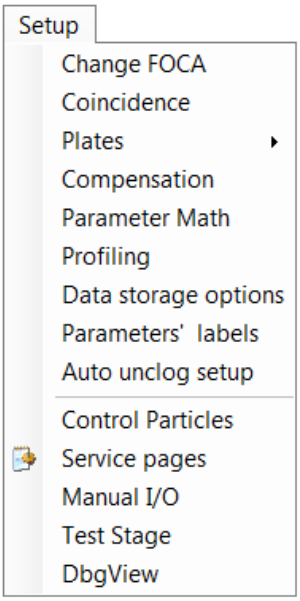


Figure 14 Screen Image Menu

9.12 RECENT EXPERIMENTS

Clicking on display Recent Experiments displays the last 4 experiments run on the instrument.

10 SETUP MENU FEATURE



tup Menu

10.1 CHANGE THE FOCA (FLUIDICS AND OPTICS CORE ASSEMBLY) MODULE

Click on this feature before changing the **FOCA** module. A dialogue box will appear prompting user to change the FOCA module. Upon clicking OK, the system will sense which FOCA module is in place and immediately import the appropriate instrument settings contained in the current experiment file.

The available FOCA modules are:

250um: for use with objects up to 200um in diameter,
500um: for use of objects up too 400um in diameter,
1000um: for use of objects up to 800um in diameter,
2000um: for use of objects up to 1500um in diameter.

10.2 COINCIDENCE

10.2.1 INTRODUCTION TO COINCIDENCE

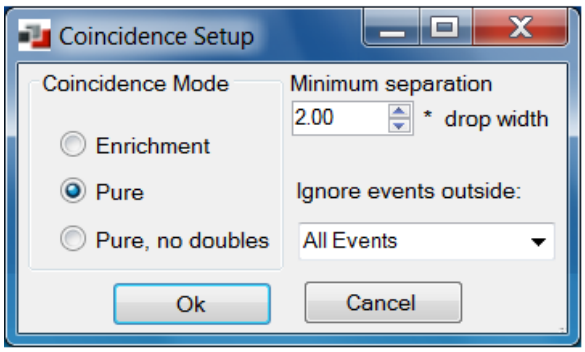


Figure 17 Coincidence Setup

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.

10.2.2 ENRICH MODE—NO COINCIDENCE CHECK

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

ENRICH-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.

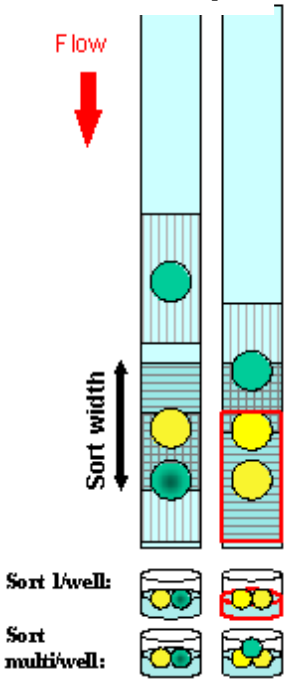


Figure 16 Enrichment Mode

ENRICH-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.

10.2.3 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).

PURE-Single object sort:

If an event is detected either within the minimum drop separation or within the drop delay behind the object, the 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, in column H, a green bead was within the drop delay causing the yellow bead to be rejected. In column I, two yellow beads are close enough in the sample stream to prevent either from being dispensed singularly. However, the tertiary yellow bead in column J has no coincidental event within minimum drop separation or sort delay; therefore it will be dispensed as a single object.

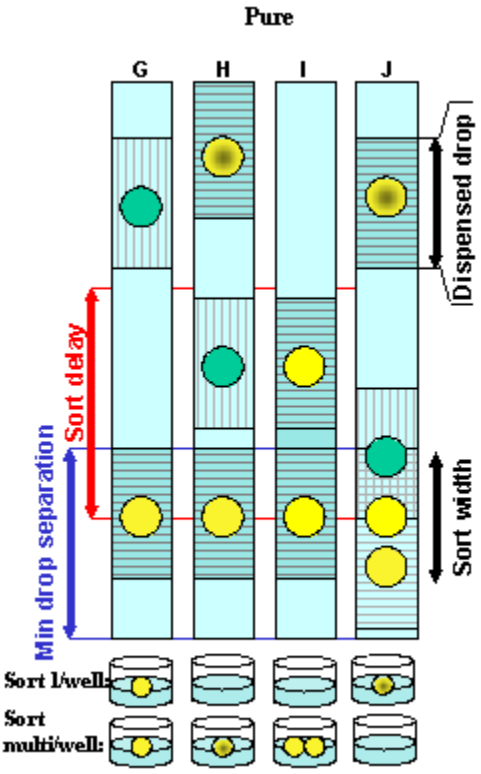


Figure 18 Pure Mode

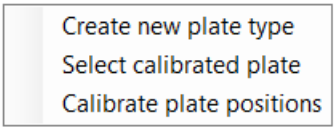
PURE-Multiple object sort command:

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, two sortable yellow beads may be coincidental to each other, preventing single dispensing, however without a nonsortable event within the sample stream both will be dispensed within a single drop. Since all visible objects are analyzed for coincidence, the system cannot dispense a contaminating event even if it lays "hidden" within the sort width of sortable objects. In column J, All three beads are analyzed for coincidence. Because the green bead is nonsortable, both yellow beads would be rejected for accurate dispensing. .In the region dispensed drop it is presumed that there is no nonsortable event behind the sortable event in column H therefore the event will be dispensed.

10.2.4 TO SET COINCIDENCE:

1. Select a Coincidence mode to use.
2. If using Pure mode, set the Minimum drops separation as a function of Sort width. Minimum drops separation should be equal to or 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 of 1.0 (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.

10.3 PLATES



This feature allows the user to:
1) Create, calibrate and store a new plate,
2) select a calibrated plate,
3) Calibrate plate positions of an existing plate

Options

10.3.1 CREATE A NEW PLATE

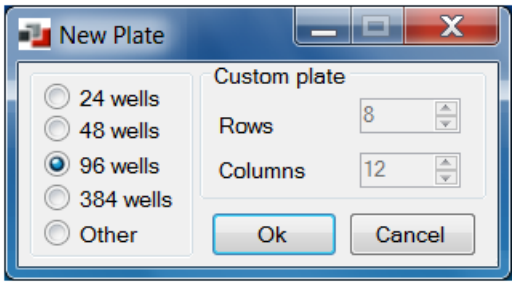
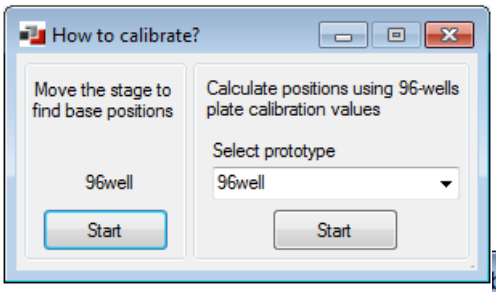


Figure 20 New Plate

Select this feature to define a new plate format.

Input the NEW PLATE format. Available options are standard 24, 48, 96, or 384 well formats. Or select other to define a custom plate format with a standard plate footprint. Input the number of rows (usually lettered along the plate's short axis) and columns (usually numbered along the plate's long axis) into the corresponding fields in the dialogue box. Then click OK.



Software will ask for input on HOW TO CALIBRATE the new plate.

Move the stage to find base positions will allow user to move the stage to coordinate locations in order to find the correct coordinates for the new plate. Or the user can instruct the instrument to *calculate positions using 96-wells plate calibration values.*

e?

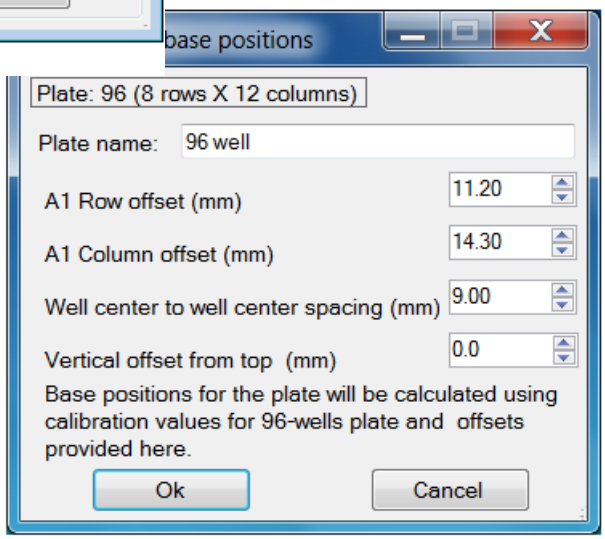


Figure 22 calculate Base Positions

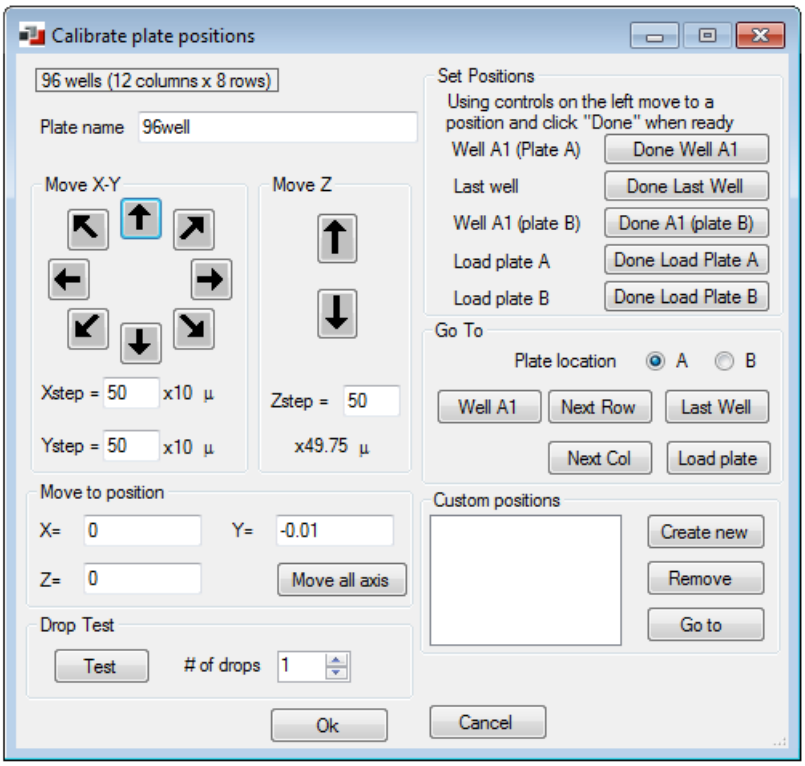
Clicking the START button under CALCULATE BASE POSITIONS *using 96-wells plate calibration values* option will bring up the dialogue box (at right). User must provide a name for the new plate and measurements pertaining to the calibrated 96 well plate and offsets (differences between 96 well plate and the new plate). To make changes input appropriate values in each field or use the arrow keys next to each field to increase or decrease the value to the correct number. Select OK button to implement the changes.

Clicking the START button under the *Move the stage to find base positions* will bring up the dialogue box allowing user to re-coordinate the well locations of a new plate. (Dialogue box at left)

To use **Calibrate plate positions**:

1. Position the new plate on the left side of the plate holder on the stage. Make sure ct plate layout (X well) information. This information is located at the top left corner of the dialogue box. If this is not correct, click the CANCEL button and select Create a new plate option under the Plates feature in the SETUP menu to set up the new plate with the correct number of columns and rows.

2. If the correct plate layout is displayed, input a name to identify this new plate in the Plate name field at the top of the dialogue box.
3. Align well A1 by selecting the Well A1 button under the Go To heading. The stage will move to the coordinates currently saved as those pertaining to the center of well A1.



4. Instruct the stage to move along the x axis in order to bring the center first well of your plate under that nozzle. The user can do this in two ways either by inputting new coordinates in the X field and click MOVE ALL AXIS button to initiate the change or by using the arrow buttons at the top of the dialogue box to move the stage in small incremental movements to the desired location. (The user can also change the incremental step movements to make larger or smaller movements each time an arrow button is pushed by adjusting the value in the Xstep, Ystep, and Zstep fields individually).
5. Check alignment by clicking the TEST button under Drop Test heading to dispense x # of drops into/onto your plate.
6. Continue to make changes in Y and Z axes until the first well of the plate is directly below the nozzle and Drop Test deposits a drop directly in the center of the well to your plate.
7. Store this set of coordinates as the new calibrated A1 position. Do this by locating the Done button directly next to the Well A1 field under the Set Positions heading at the top right of the dialogue box.
8. Next choose Last Well button under the Go To heading. As done with A1 position, move coordinates until the last well is directly centered under the nozzle. Set the Position of the last well by clicking the Done button next to the Last well position.
9. Continue setting positions for Load plate A and Load plate B in a similar manner.
10. Once the centers of A1 and the last wells have been defined and stored with the new plate's name click OK to accept the change. At this point the instrument will calculate the necessary step movements to cover the distance between wells so that each well location is correctly coordinated. The newly defined plate will now be a plate template option during sorting.

10.3.2 SELECT CALIBRATED PLATE

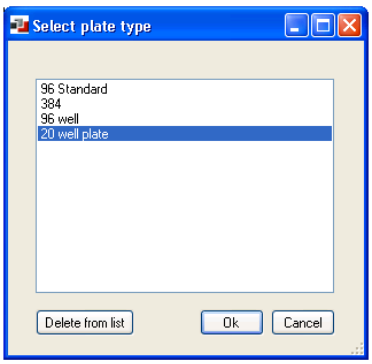


Figure 24 Select Plate Type

Allows user to select a predefined plate to use as a sorting template. Choose the correct plate and click the OK button to open the template. User may define the well locations, number of objects to dispense and well order to dispense. Additional information can be found under section 11.4 PLATE TEMPLATE as a feature in the VIEW menu.

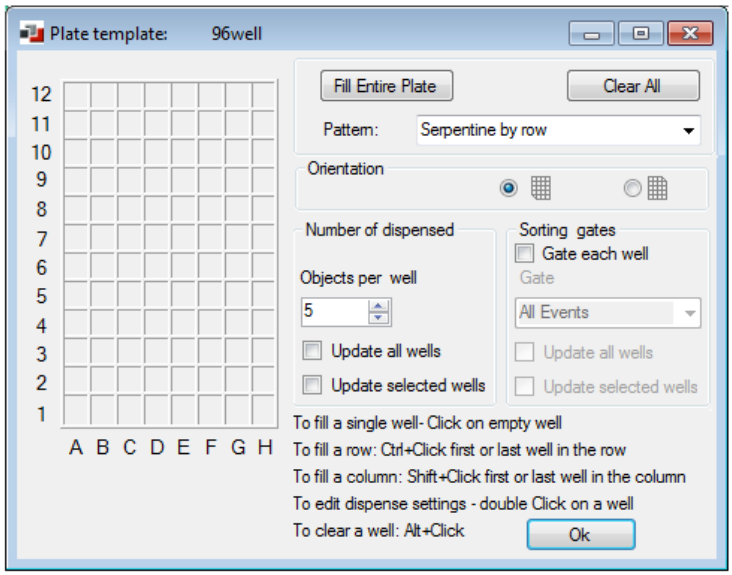


Figure 25 Plate Template

10.4 COMPENSATION

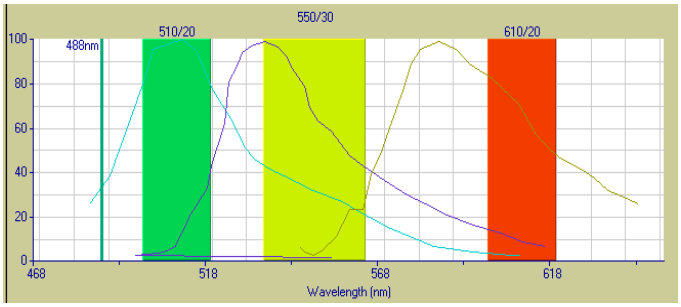


Figure 26 Compensation Graph and Table

The BioSorter instrument uses multiple filters to best capture individual color fluorescence; however, there may 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 through 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 BioSorter instrument 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 FlowPilot software makes this adjustment in real time and is used as criteria for a sorting decision.

To use:

1. Open the COMPENSATION SETUP dialogue box from the SETUP 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. Locate the Target and Source channels to be adjusted. For example, Target channel (yellow) should be reduced by X% of the Source (green) signal. The percentage value appears in the box to the right of the sliding bar.
3. Click Apply compensation to profiles if applicable.
4. 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 unless data storage options are modified to record the compensated values. Compensation settings will be saved in the stored template, CSV file.

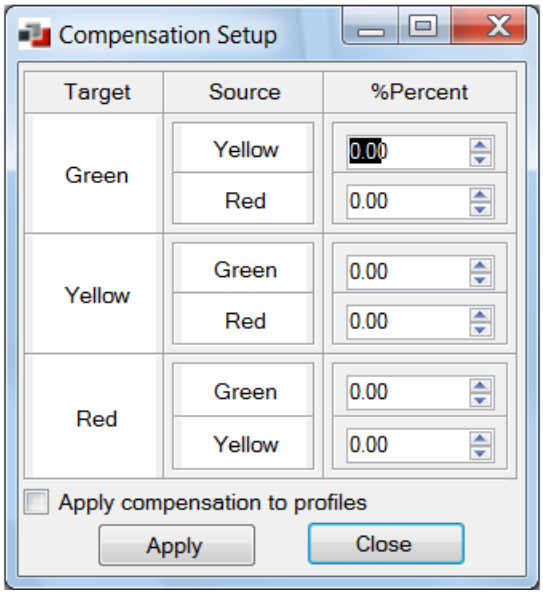


Figure 27 Compensation Setup

10.5 PARAMETER MATH

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

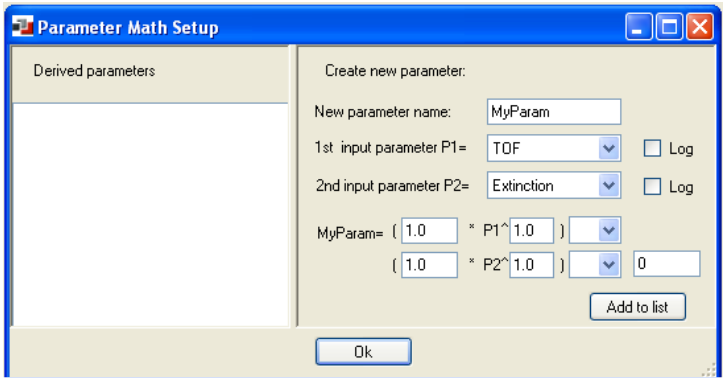


Figure 28 Parameter Math Setup

To use:

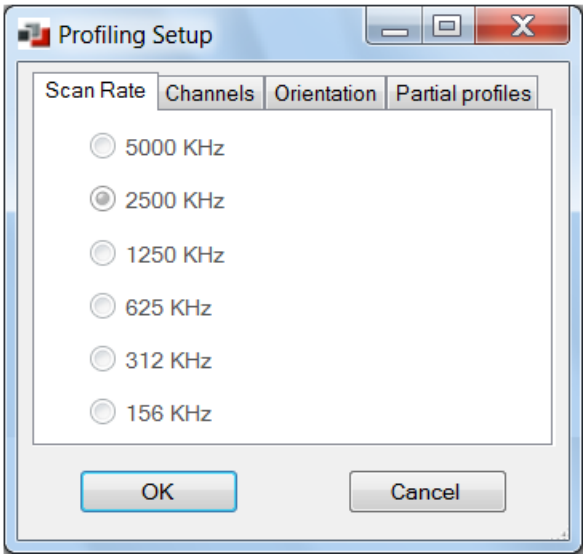
1. Open the PARAMETER MATH item under the SETUP pull down menu.
2. Locate the New Parameter name window and define a name for the new parameter.
3. Define 1st and 2nd input parameters by using the pull down menu tab of available parameters.
4. Define the new function using the manipulators available in the MyParam=fields.
5. Click the ADD TO LIST button. The new parameter should now appear at the left under derived parameters heading.
6. Click OK to implement the changes. At this point the new parameter math titles will appear as available parameters for use in histogram and dot plot axes.

NOTE: Stored data in txt and lmd files will contain raw data, not the converted parameter functions employed during data acquisition.

10.6 PROFILING

The profiling feature is fully characterized in its own chapter see Section 16 PROFILING FEATURE.

10.6.1 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 5000KHz 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 2500KHz, but user can change the setting any time system is not sampling. Also note that the stored txt file of data contains a summary line indicating the scan rate used during data acquisition.

10.6.2 CHANNELS

User can select which channels to activate during profiling. Click on the individual channels to activate. A checkmark next to the channel indicates it is active.

User may also change the color of the display of each profiled channel. Click on the colored box next to the profile channel and select a new color from the available choices or define a custom color. Click OK to implement the change.

10.6.3 ORIENTATION

Allows user to set conditions for Profiler to identify the orientation of a profiled object and align the displayed profile according to assigned orientation. See section 16PROFILING FEATURE for more information about setting up this option.

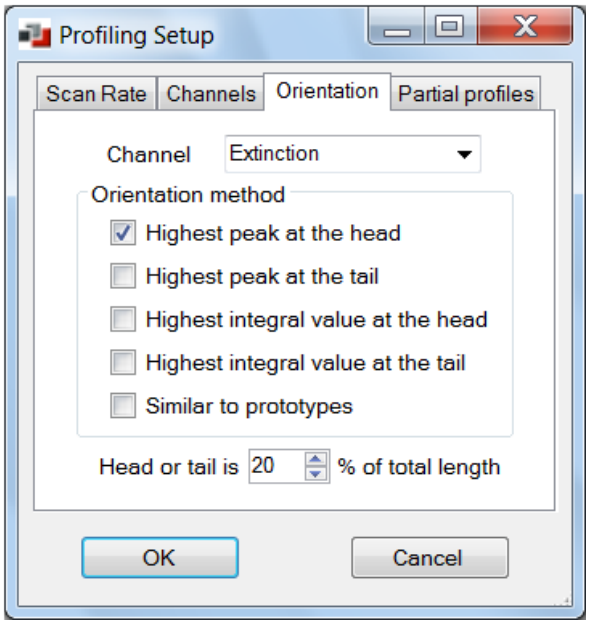


Figure 29 Profiling Setup

CHANNEL

User may select the channel in which to perform orientation evaluation.

ORIENTATION METHOD:

User may select one set of conditions to orient the object including: Highest peak at head, Highest peak at tail, Highest integral value at the head, Highest integral value at the tail, or to apply the orientation based on profiler prototypes.

HEAD OR TAIL % OF TOTAL LENGTH

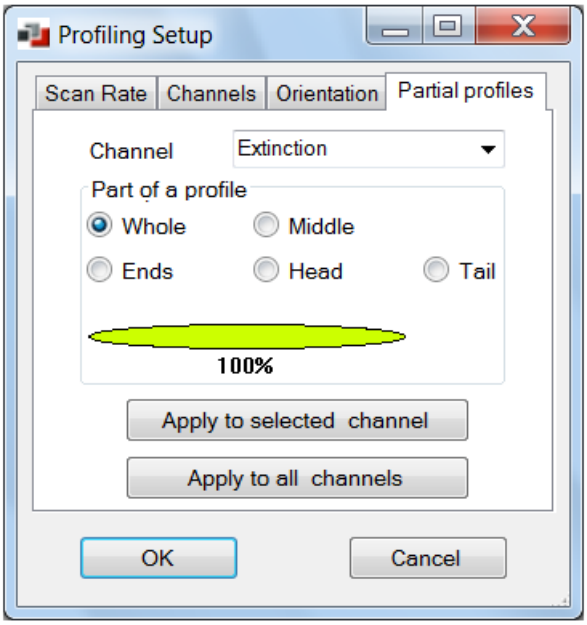
User may change the percentage of the total length defined as head or tail for orientation evaluation.

10.6.4 PARTIAL PROFILES

User may choose to activate extinction and/or fluorescence evaluation over a part of the object profile.

CHANNEL

User may select one or more channels in which to assign criteria for partial profile analysis.



PART OF PROFILE

User may choose one area to evaluate features of the profile, including:

Whole profile

Identifies features across entire profile.

Middle of profile

Identifies parameter features between % of ends, ie: middle of the profile.

Ends of profile

Identifies parameter features at both ends of the profile (contained within the user define % of profile).

Head of profile

Identifies parameter features on only the front end of the displayed profile.

Tail

Indentifies profile features on only the far end of the displayed profile.

Note: Head will always be the beginning of profile, and tail the end of the profile UNLESS orientation criteria is used during partial profile analysis.

APPLY TO SLELETED CHANNEL / APPLY TO ALL CHANNELS

User must select whether to apply the partial conditions to only the selected channel or ALL channels.

10.7 DATA STORAGE OPTIONS

10.7.1 FILES STRUCTURE

User can choose the volume of content stored in each file.

SINGLE FILE OF EACH TYPE

Indicates there will be a single txt and lmd file for the entire data acquisition.

MULTIPLE FILES, ONE PER WELL

Indicates each well will have an individual file for txt and lmd files.

MULTIPLE FILES, X EVENTS PER FILE

Allows user to preselect the volume of data so that each file contains x # of events.

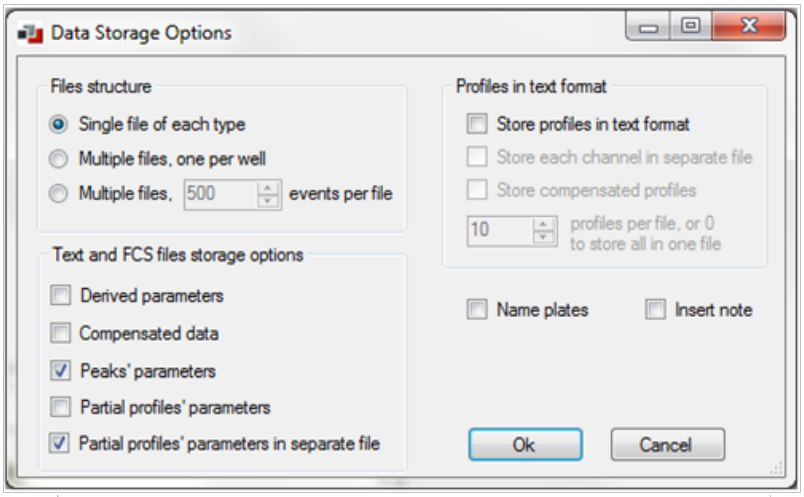


Figure 30 Data Storage Options

10.7.2 TEXT AND FCS FILES OPTIONS

STORE DERIVED PARAMETERS

Option allows user to store parameter math derived values.

STORE COMPENSATED DATA

Option allows user to store compensated values instead of the raw values.

STORE PEAKS' PARAMETERS

Option directs storage of profiling parameters such as highest peak height, peak width at user defined level, and peak count when feature is active.

PARTIAL PROFILES' PARAMETERS

Activated, this option will store the integral values calculated from partial profiles.

PARTIAL PROFILES' PARAMETERS IN SEPARATE FILE

Activated, this option will store the peaks' parameters calculated from partial profiles in an additional file.

10.7.3 PROFILES IN TEXT FORMAT

User can choose if they would like to **store profile in text format**. Option stores profiling values (changes in fluorescence intensity as the object is scanned) in text file. See profiling chapter for more information on this feature. A check mark in the box indicates this feature is selected.
Once profile storage as text file is selected, user can choose to store profile data in a number of ways by placing a checkmark in the box to activate the available option:
Store each channel in a separate file, otherwise all active profiling channels will be stored in the same file.
Store compensated profiles, otherwise default is set to store profiles original values (uncompensated).
User can choose the number of object profiles to be contained in each text file so as not to generate large cumbersome text files. Selecting 0 directs software to store all object profiles in a single file.

NAME PLATE

User may activate this feature to include a plate name in the stored data file.

INSERT NOTE

User may activate this feature to include a note in the stored data file.

Click OK button to implement changes to data storage.

10.8 PARAMETERS' LABELS

User may redefine parameter labels as is necessary for use of specific lasers, filters, stains, dyes, markers, etc.

To Use:

Open the Parameters' labels heading in the Setup menu. The EDIT LABELS dialogue box will appear. Select the parameter to be modified, input the new label and click OK to implement the change. Once done the new label should appear instead of the original in all parameter menu items.

Figure 31 Edit Labels

10.9 CONTROL PARTRICLES

Control particles allows the user to run a standardized bead sample to verify instrument performance. Instrument settings are preset for each flow cell to allow for consistency each time control particles are run. It is recommended that control beads be run every time the flow cell module is changed and at the beginning of each day's use of the instrument. See section 5.4 RUNNING CONTROL PATRICLES in the operations section of the manual.

To use:

1. Select Control particles from the SETUP pull down menu.
2. Notice 5 histograms and a profiler graph pop onto the software screen.
3. Fit a sample cup or tube of control particles onto a clean instrument and select acquire.
4. Reduce sample pressure as is necessary to achieve a slow narrow sample stream. Acquisition rate should be around 10 objects per second.
5. Erase data after changes have been made and collect at least 500 events to perform statistical analysis for bead uniformity.
6. To return to FlowPilot software screen, unselect the control particles option from the setup menu.

10.10 SERVICE PAGES AND MANUAL I/O

Service pages and Manual I/O are areas of the instrument software used by Union Biometrica service personnel. These contain factory preset values. Any modification to files contained in this section may result in problems running the instrument.

10.11 TEST STAGE

This feature is used by service person to instruct the stage to perform a scripted exercise to test the stage functions.

10.12 DEBUG VIEW

This feature may be activated to log each instrument command. It may be used by a service person to obtain a history of the instrument's current performance and/or use.

11 VIEW MENU FEATURES

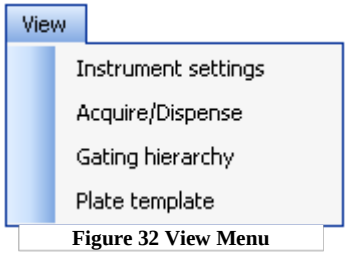


Figure 32 View Menu

Features contained in the VIEW menu relate directly to how the data is captured and settings for analysis and dispensing.

11.1 INSTRUMENT SETTINGS

11.1.1 EXTINCTION DETECTOR

Extinction is the optical density determined for each objects as it passes through the path of the laser. It can be thought of as the shadow cast on the extinction detector. Individual objects cast different shadows as a function of their opacity and internal structure and a wavelength of the laser is used to capture this information. For these reasons the BioSorter has made available several extinction detector filters to optimize the extinction signal for different samples and applications.

The power displayed is the amount of light 'seen' at the extinction detector. The Power at the extinction detector is displayed in the INSTRUMENT SETTINGS dialogue box to allow the user to monitor and make adjustments to fulfill specific sample requirements. A reduction in the laser power will reduce the power registered at the extinction detector. Additionally, there are several filters that may be placed in front of the extinction detector that will alter the amount of light reaching the extinction detector. A reference to the laser power at extinction detector is recorded in the text file in the summary section at the end of the file.

There are several ways to change the laser power at the extinction detector:

Reduce or increase the source laser power. Reduction or increase will have a similar effect on the extinction detector.

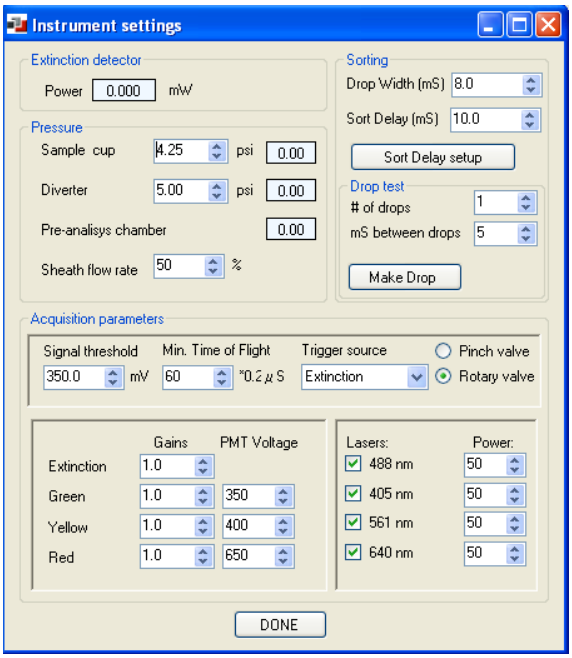


Figure 33 Instrument Settings

To change extinction settings:

1. Identify which filter is in place in front of the extinction detector. This filter is located just to the left of the flow cell module. Then locate the LASERS: heading at the bottom right of the INSTRUMENT SETTINGS dialogue box.
2. Input the new laser power or use the arrow keys next to the appropriate laser power field to alter the laser power applied to that laser then hit the enter key. At this point the user should see the POWER displayed at the Extinction detector has changed. If the EXTINCTION DETECTOR POWER did not change, toggle the check mark next to the laser to apply the change.

Another way to change the extinction signal is to change the filter in front of the extinction detector. Laser should be turned off before the extinction filter is changed. Then changes to the source laser can be modified to produce the desired effect.

11.1.2 PRESSURE

Pressures are electronically monitored and regulated. The left window allows user access to increase or decrease the pressure applied to the sample cup and diverter by increasing the integers displayed in these pressure status fields. The instrument then requires a brief moment to respond and regulate the change in pressure. The right window indicates the actual pressure the instrument is reading.

User may alter the sheath flow rate by changing the flow percentage displayed in the sheath flow rate window. Always toggle the sheath valve once a change has been made to accept and apply the change. Then monitor the volume output to ensure correct output has been achieved.

11.1.3 SORTING

DROP WIDTH

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

DROP DELAY

Indicates the amount of time, in milliseconds, from analysis of the object to the sort command. The value for SORT DELAY is Flow Cell Module and sample dependent. By performing a test dispense or sort delay setup, the appropriate delay can be checked.

SORT DELAY SETUP

Allows user to test different sort delay conditions automatically in order to determine the appropriate delay setting required for their sample.

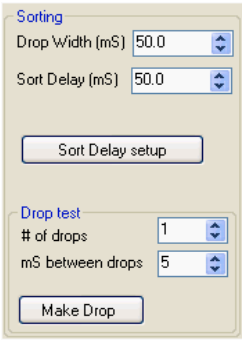


Figure 34 Sorting Portion of Instrument Settings

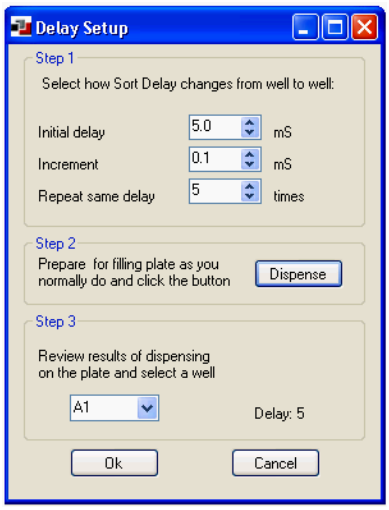


Figure 35 Delay Setup

To use:
Click on the Sort delay setup button. A Delay Setup dialogue box will be opened. Follow the steps as indicated.

Step 1:Define the Initial Delay setting to begin testing (measured in milliseconds.

Define the Increments of delay increase to test.

Define the number of wells to repeat the same delay.

Step 2:Prepare dispensing. Make sure the region and number to dispense is set up correctly then press the DISPENSE button to begin dispensing the various delays to test.

Step 3: View the dispensed objects under the microscope to identify the location of the well with accurate dispensing. Input that well location into the 'select a well' field. The delay corresponding to that well will be displayed to the right DELAY: #.

Click OK when done to close the Delay Setup window. The determined delay is automatically imported into the delay field of the active instrument settings window.

DROP TEST

Allows user to test the formation of the dispensed drop by directing the system to deposit a drop of sheath fluid directly below the sort nozzle.

To use:
Insert number of drops to make.
Input the timing (in milliseconds) between drops. Click the MAKE DROP button to initiate test drop formation.

11.1.4 ACQUISITION PARAMETERS

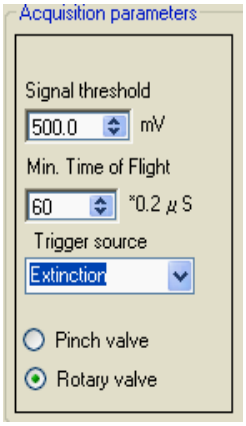


Figure 36 Acquisition Parameters

SIGNAL THRESHOLD

Sets the position of the 0 channel 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 trigger source selector.

MIN. TIME OF FLIGHT

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. It can be used to eliminate analysis of debris that may impede sorting.

TRIGGER SOURCE

User may choose the source of signal to trigger detection. User may select extinction, PMT1 (green), PMT2 (yellow), or PMT3 (red).

PINCH VALVE OR ROTARY VALVE

Indicates which valve type is currently in use. The Rotary valve is utilized for the smaller sample tubing, while the larger tubing is connected to the Pinch valve.

11.1.5 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 100 times amplified. When attenuating signals, the trigger Threshold may need to be adjusted as well.

NOTE: With higher gain comes a compromised responsiveness. A gain greater than 10 risks causing a negative effect in relation to the responsiveness of the system.

EXTINCTION

Figure 37 Gain Display

Is a measure of 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. Extinction can also be referred to as optical density.

PMT 1 (GREEN)

Specifies a (changeable) filter on the first PMT channel of the system. Standard configuration employs a green filter of 510 nanometers optimum with a band width of 23 nanometers. Emission can be collected from various fluorochromes.

PMT 2 (YELLOW)

Specifies a (changeable) filter on the second PMT channel of the system. Standard configurations employs a yellow filter of 545 nanometers optimum with a band width of 25 nanometers. Emission can be collected from various fluorochromes.

PMT 3 (RED)

Specifies a (changeable) filter on the third PMT channel of the system. Standard configuration employs a red filter of 610 nanometers optimum with a band width of 20 nanometers. Emission can be collected from various fluorochromes.

11.1.6 PMT VOLTAGE

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

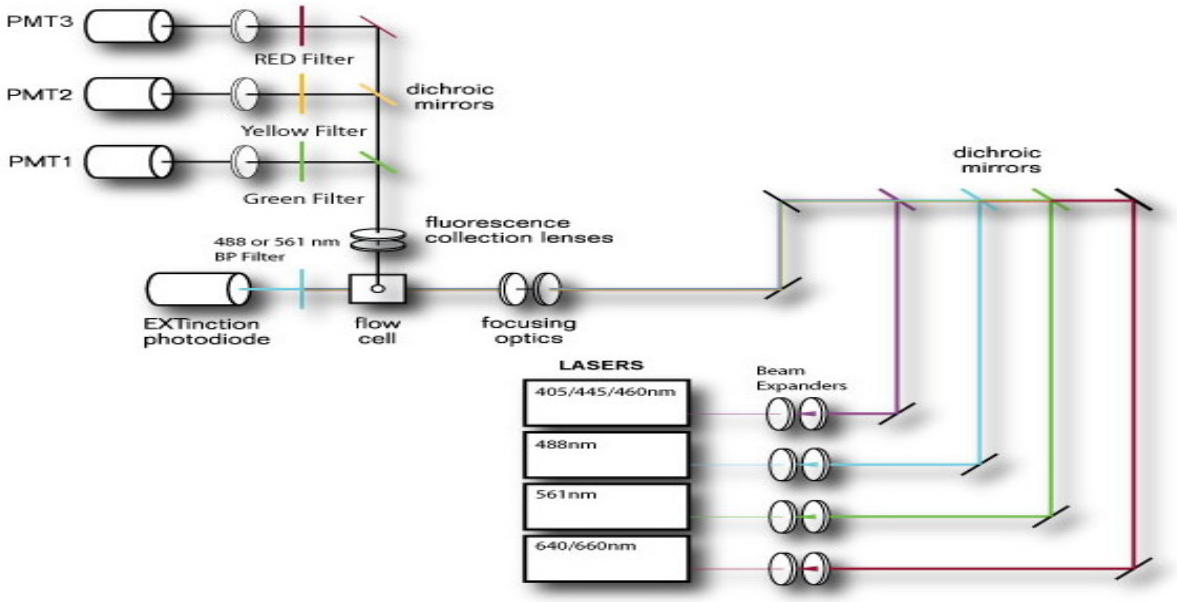


Figure 38 Simple Schematic of Optical Path

determine a baseline PMT setting.

11.1.7 LASER CONTROL

Control activation and laser power for each laser on the system. A checkmark indicates the laser is running. Input an integer in each of the laser fields to define the power of each laser used. **NOTE: laser power is very important in how individual object extinction and fluorescence is detected.** For this purpose the laser power is recorded in the text file of each stored sample and can be reviewed if necessary.

11.2 ACQUIRE/DISPENSE

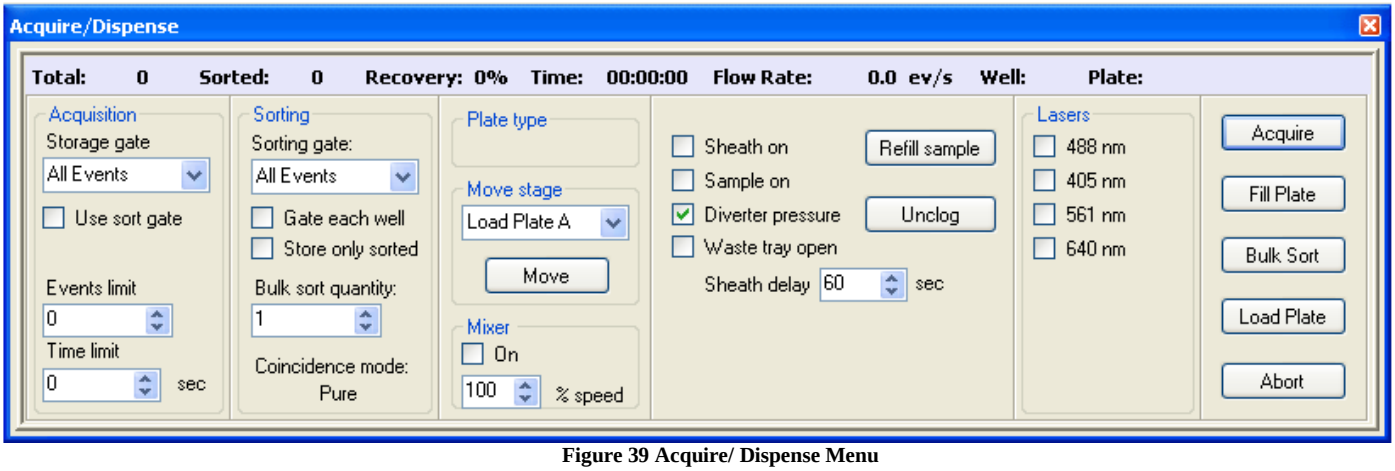


Figure 39 Acquire/ Dispense Menu

11.2.1 DATA SUMMARY

TOTAL

Displays the total number of acquired events

SORTED:

Displays the number of objects dispensed.

RECOVERY:

Displays the % of objects that were dispensed as a portion of all objects that fulfill sort criteria.

TIME:

Displays the amount of time that has passed since user input file name for data storage.

FLOW RATE:

Displays the acquisition rate of objects during data acquisition as a # of events per second.

WELL:

Displays the current location of the stage based on the current plate template being used.

PLATE:

Displays the Plate number (in sequential order) currently being dispensed (since the instrument was last turned on).

11.2.2 CONTROL BUTTONS

ACQUIRE

Clicking the ACQUIRE button will initiate data acquisition. A dialogue box will open directing the user to store the data they are about to acquire. Clicking cancel will allow data acquisition without storage. ACQUIRE button changes to read PAUSE during sample acquisition.

Figure 40 Control Buttons

FILL PLATE

Click the FILL PLATE button to begin sorting to a multiwell plate as defined in Section PLATES. 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.

BULK SORT

Click the BULK SORT button for continuous bulk sorting of 1 to 50,000 objects. BULK SORT is used for sorting the specified number of events as defined in the bulk sorting field in the Sorting heading of the ACQUIRE/DISPENSE box.

LOAD PLATE

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

ABORT

ABORT BUTTON will immediately abort the current instrument function. This functions to stop whatever the instrument is currently doing. Clicking once will stop acquisition as well as close any fluid valves that were open.

11.2.3 ACQUISITION

STORAGE GATE

User can select to store all events or a portion of all events by selecting a gate containing the events to store. Note: all other events will not be stored and cannot be viewed during review data mode.

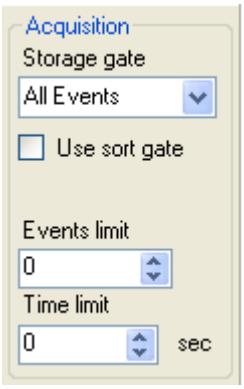


Figure 41 Acquisition Limits

USE SORT GATE

When an X is placed in this box data will be stored on the specified sort gate.

EVENTS LIMIT

User may choose to automatically stop data acquisition once a certain number of events are acquired. User can input any number of events in the field. Once this number is reached data acquisition will be stopped, sample valve closed, and sheath stopped.

TIME LIMIT

User may choose a time limit to place on data collection. Input a time limit (in seconds) at which point data acquisition will be stopped, sample valve closed, and sheath stopped.

NOTE: If system is dispensing at the point the Event or time limit has been reached, system will continue with the acquisition and dispensing until the remainder of wells (or bulk #) is reached.

11.2.4 SORTING

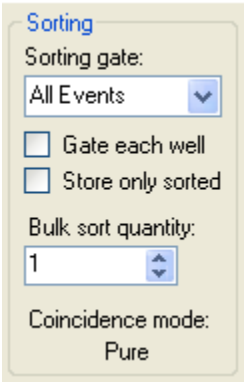


Figure 42 Sorting Limits

SORTING GATE

User selects a defined gate containing criteria for dispensing.

GATE EACH WELL

User may place an X in this box which will promote the plate template to allow access to the SORTING GATES conditions. From these conditions a user can select to sort specific regions to specific wells.

STORE ONLY SORTED

User may place an X in this box to store only data from dispensed objects. All other object data will be lost.

BULK SORT QUANTITY

Bypassing the sort template, user may input a number of objects (within SORTING GATE) to dispense directly below the flow cell nozzle. User may find Move to plate location useful to bring stage under the nozzle for bulk dispensing to a receptacle.

COINCIDENCE MODE

Displays the current coincidence mode employed: Enrichment or Pure. See section 10.2 COINCIDENCE for more information on this feature.

11.2.5 LASERS

Displays which lasers are active during data acquisition. These are automatically turned on when ACQUISITION is initiated.

11.2.6 PLATE AND STAGE

PLATE TYPE

Displays the name of the plate template currently in use.

PLATE POSITION ON STAGE

Allows user to select the left only, right only, or Left and Right stage positions during stage use, i.e. dispensing.

MIXER

Figure 43 Mixer Setting

Allows user to manipulate the speed of the mixing paddle/probe. A checkbox indicates the mixer is on. Input an integer into the speed field or use arrow keys at the right of the field to make mixer speed changes.

User may also choose to activate a mixer oscillation setting by inputing a timing frequency (every X seconds) to change mixer direction. 0 indicates the mixer does not change direction.

11.2.7 MANUAL CONTROLS

SHEATH AND SAMPLE VALVES

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 MANUAL CONTROLS. A checkmark in the field indicates the valve is open.

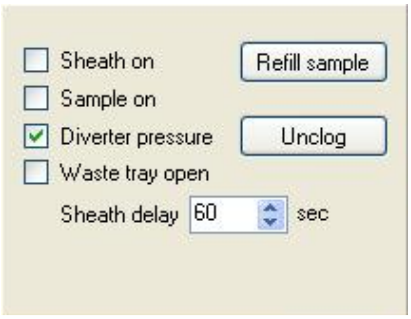


Figure 44 Manual Controls

DIVERTER PRESSURE

User can manually control the operation of the diverter valve by checking (air is supplied to divert the waste stream) and unchecking (air turned off waste not diverted) the box.

WASTE TRAY

Place an X in this field to command the waste tray to move into position to allow dispensing. This is particularly useful to check the sheath flow.

REFILL SAMPLE button

Once clicked, the sample valve is closed and the sample cup depressurized, allowing the user to open and refill the sample cup. Press the DONE FILL button when finished. This will repressurize the sample cup before it allows user to open the sample valve again.

UNCLOG button

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

SHEATH AND SAMPLE DELAY

This is the time (in Seconds) that the sheath valve will remain open (sheath flowing) after acquisition has ended. During successive plate dispensing, user may prefer to keep the sheath running while plates are changed and dispensing is again initiated.

11.2.8 MOVE STAGE

Select a stage position from the pull down menu then press the MOVE button to direct the stage to move to the desired location.

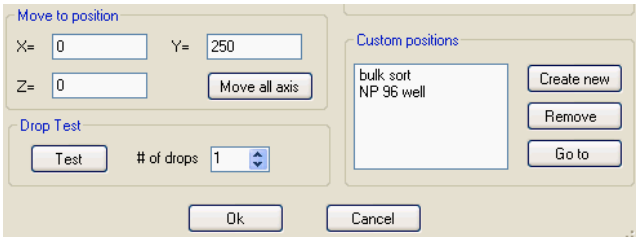


Figure 45 Custom Plate Positions

To define a stage position:

Open the calibrate Plate positions feature under the Setup menu heading.

Move the stage using arrow keys or axis coordinates to desired plate position (use MOVE ALL AXIS button).

Once stage is in the desired location, click the CREATE NEW button under the custom positions heading, and define a name for this new position.

Click OK button to accept the changes and exit the calibrate plate positions Setup feature.

11.3 GATING HIERARCHY

Displays a flow chart of the gating priority.

11.4 PLATE TEMPLATE

Allows the user to view the current plate template.

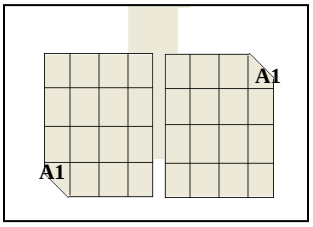


Figure 46 Stage Format for Orientation

To Use:
With the mouse click on the well to be filled and input the # of objects to dispense to that well. Repeat this for each well to be filled. User can also use the shortcut options at the right half of the template window. These are listed below:

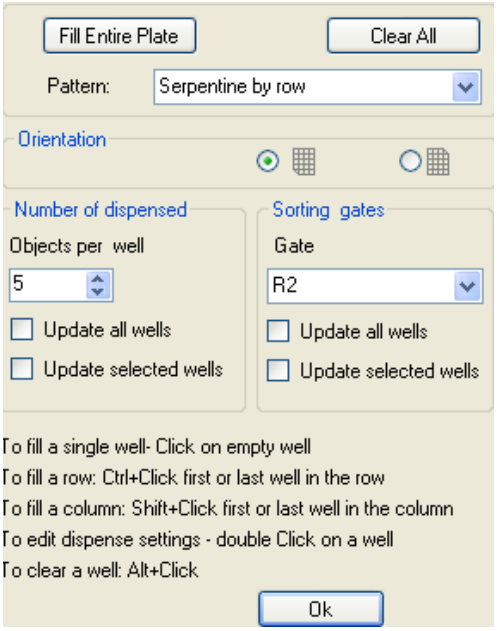


Figure 47 Filled Plate Template Options

FILL ENTIRE PLATE

Clicking this button will identify every well to be filled with x number of objects defined in the Objects per well field.

CLEAR ALL

Clicking this button will erase settings for all well locations.

PATTERNS

Allows user to select a pattern for filling the wells in a potentially more efficient manner.

ORIENTATION

User may designate the orientation of the plate placed on stage (left) position A. Corner cutout refers to well location A1 (see Figure 45 above).

NUMBER OF DISPENSED

This field allows users to change the number of objects to be dispensed to the wells.

Objects per well
Input a number of objects to dispense into each well.

Update all wells
Toggling this field will change ALL filled well locations to the number of objects per well currently displayed.

Update selected wells
Toggling this field will change only the currently highlighted wells to the number of objects per well currently displayed

SORTING GATES

This field allows users to change the region being dispensed to the wells.

Gate
Input a gate region to be dispense into each well.

Update all wells
Toggling this field will change ALL filled well locations to the active gate selected.

Update selected wells
Toggling this field will change only the currently highlighted wells to the active gate selected.

Fill a single well by clicking on an empty well
Fill the whole row by holding down the Ctrl key and clicking first or last well in the row.
Fill the entire column by holding the Shift key and Clicking the first or last well in the column.
Edit the dispense settings by double clicking on the well to modify **Clear a well** by holding down the ALT key and clicking the well to be erased.

User can make changes to the template set define or configure a new plate type, etc using features described in the SETUP menu heading, plate template.

12 LAYOUT MENU FEATURES

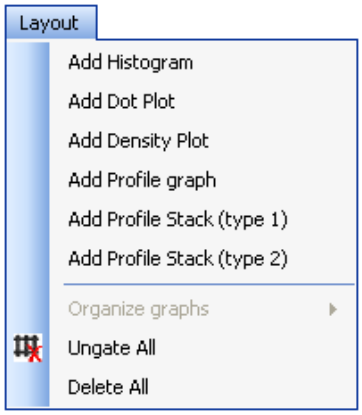


Figure 48 Layout Menu

FlowPilot software is customizable in how data is presented on screen. The user may choose up to 32 data windows and regions across these windows.

12.1 HISTOGRAM

Each histogram displays the frequency of a single parameter value within a data set by displaying the values in data columns. X axis displays the measured data parameter (value at the far right indicates max value represented on the plot), Y-axis displays the count of each data point in the column (value at top indicates the max count number represented in the column).

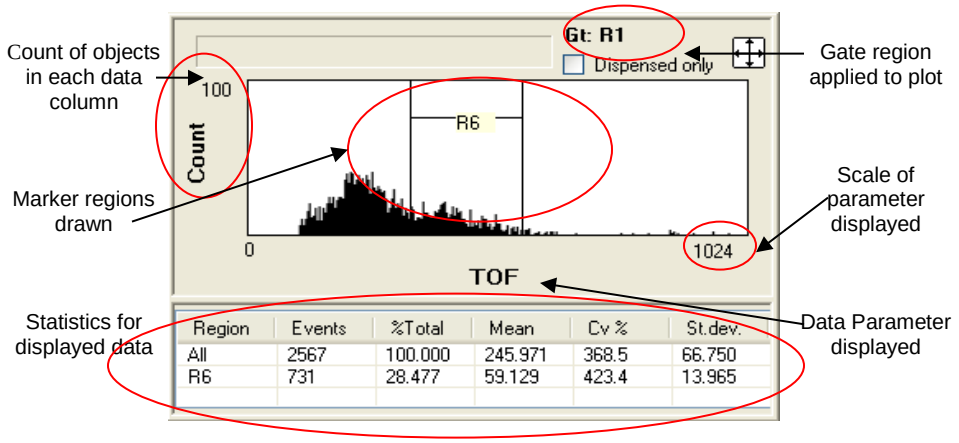


Figure 49 Histogram Displaying Time of Flight

12.1.1 MODIFY THE HISTOGRAM

Right click on the X-axis label to change the parameter being presented. View only dispensed objects by placing an X in the box at the upper right corner labeled: Dispensed only. User may choose to view all dispensed objects or a particular well where objects were dispensed.

Right click within the histogram to bring up the options menu.

RESCALE HISTOGRAM-

X-axis:
STANDARD SCALING-check the max scale to represent your data in histogram mode.
USE CUSTOM SCALE-input a nonstandard max scale setting for the histogram plot.
OFFSET value-X axis will be changed so that the Offset value is displayed at the far left edge of the window and the custom or other max scale is displayed at the far right of the graph.
LOG SCALE-plot data in 1 to 5 decades of log scale.

Y-Axis:
Change the MAX COUNTS displayed in each 'column' of data.

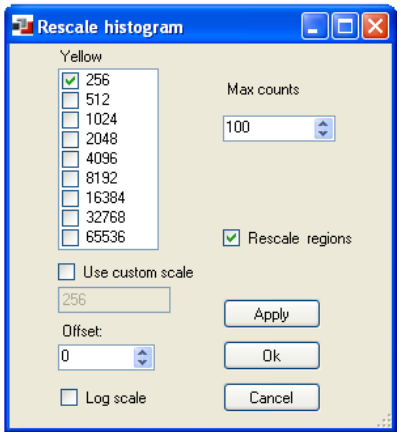


Figure 50 Rescale Histogram Display

A check mark applied in RESCALE REGIONS instructs the software to automatically resize any bar regions according to your defined scale factors.

Click APPLY, and then OK to accept the scaling changes and exit the rescale window.

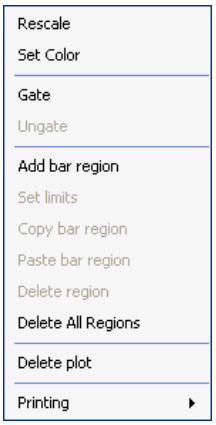


Figure 51 Histogram Options Menu

SET COLOR

Allows the user to change the display color of the histogram. Select the color of choice, and then click OK.

GATE

Allows the user to select a gating region from another layout window for display within the histogram. Select one of the gating regions from the list provided and click OK. Note that when gating, only data from the selected plot/gate are displayed on the histogram plot.

UNGATE

Removes gating criteria from the histogram, all acquired data is displayed.

ADD BAR REGION

Allows the user to select a specific region of the histogram for gating, dispensing, or display purposes. Once the bar region is created, left click and drag the marker to customize the window. Multiple bar regions may be created for a single histogram. Bar regions can be re-named by left-clicking on their label and typing the new name.

SET LIMITS

Allows user to manually input integer limits for the bar regions (end points to the bars).

COPY BAR REGION and PASTE BAR REGION

Allows user to duplicate and paste a bar region on the same or another histogram window.

To use: While hovering over the bar region, right click and select copy bar region. Move to another histogram. Right click again and select paste par region. An identical bar region will be pasted in the histogram. It is given the next consecutive region number.

DELETE REGION

Allows user to remove a bar region from a histogram. While hovering over the bar region, right click and select delete region.

DELETE ALL REGIONS

Removes all gated regions from the plots.

DELETE PLOT

Removes histogram from the sample template. Note that any gating regions in the histogram will be removed from the gating hierarchy. This may result in deleting regions that are dependent upon any bar regions or gating criteria utilized in the histogram that is deleted.

PRINTING

This feature allows the user to 1) Print the data contained within the histogram, 2) Look at the print set up conditions for the histogram, and 3) Save the histogram image as a .bmp file.

12.2 ADD DOT PLOT AND ADD DENSITY PLOT

Each Dot or Density plot displays 2 parameters where each point represents a single data point (on standard dot plot) or several events (on density plot). X and Y axes display the measured data parameter and the scale shown. Plots also may show gate/sort regions and statistics pertaining to the different regions.

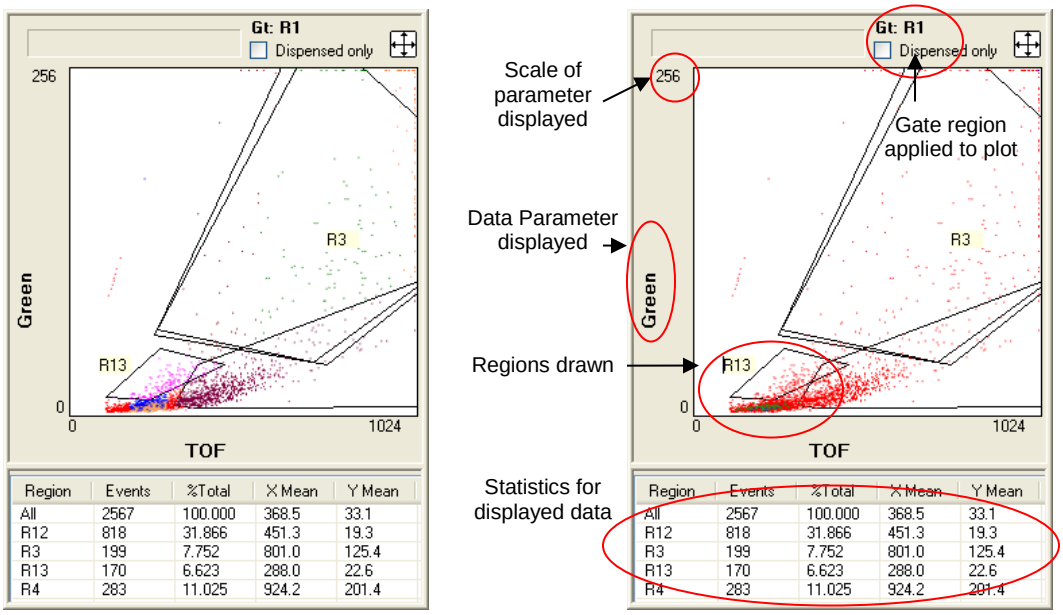


Figure 52 Representatives of Dot and Density Plots

12.2.1 MODIFY THE DOT/DENSITY PLOTS

Right click on either the X-axis or Y-axis labels to change the parameter being presented. Select from the list displaying all options including derived parameter using parameter math feature.

Right click within the dot/density plot to bring up the options menu.

RESCALE

Independent X-axis and Y-axis scaling:

STANDARD SCALING-check the maximum plot scale to represent your data in dot plot mode.
USE CUSTOM SCALE-input a nonstandard max plot scale setting for the dot plot.
OFFSET value-X/Y axis will be changed so that the Offset value is displayed at the origin of the graph and the custom or other max plot scale is displayed as the upper limits of the graph.
LOG SCALE-plot data in 1 to 5 decades of log scale.
RESCALE SORTING REGIONS instructs the software to automatically rescale all existing regions whenever scales are changed.
Click APPLY, and then OK to accept the scaling changes and exit the rescale window.

Figure 53 Rescale Dot/Density Displays

PARTIAL PROFILE

Upon activation, data will reflect calculated values based on partial profiling conditions. The displayed parameter will be changed to include ***PP*** next to the heading title.

GATE

Allows the user to select a gating region from another layout window for display on the dot/density plot. Select one of the gating regions from the list provided and click OK. Note that when gating, only data from the selected plot/gate are displayed on the dot/density plot.

UNGATE

Removes gating criteria from the histogram, all acquired data events are displayed.

DRAW REGION

Allows the user to select a specific region of the dot plot for gating, dispensing, or display purposes. To create a region, draw a polygon by moving the "+" cursor and left-clicking to drop a vertice to surround the region of choice. To close the gate, right click on the mouse and the final segment of the box will be drawn for the user. Multiple regions may be created on a single dot plot and they may overlap. Regions can be re-named by left-clicking on their label and typing the new name. This name will appear in the statistics window with the region identifier and with both the rename and region identifier in the Heirarchy display at the bottom of the software screen.

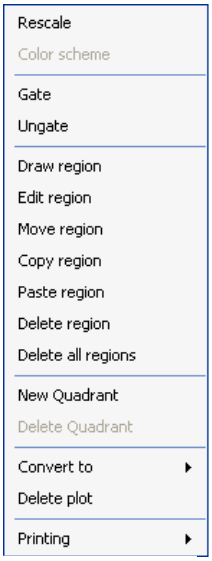


Figure 54 Dot Plot Options

EDIT REGION

Allows the user to move the points of a given gate region in order to fine-tune the gate. In order to edit the region right click on the region boundries and select EDIT REGION. The region boundaries will become dashed and a single vertex will become red indicating it is selected for modification. Move the vertex by dragging it to a new location or manually input new coordinate locations for the highlighted vertex. Click a different vertex and continue to make changes as necessary. Right click the mouse to accept the changes and exit the edit feature.

Figure 55 Coordinates Menu

MOVE REGION

Move an entire region within the dot plot without changing its size or shape. Right-click on a region boundary and select MOVE REGION to move it. The region will become filled in with hash marks. Drag the region to a new location and right click to accept the change and exit from the move feature.

COPY and PASTE REGION

Copy a region from the dot plot and paste it into another dot or density plot. Right click on a region boundary and select COPY REGION. Move to another dot or density plot to and again right click within the plot and select PASTE REGION. Note: It is possible to paste a region over the original in the same dot plot. In this case the new region will completely overlap, and obscure the original--it is best to move one of these to a new location.

DELETE REGION

Removes selected region from the dot plot. Right-click on the region boundary you wish to remove and select DELETE REGION.

DELETE ALL REGIONS

Removes all regions from the dot plot.

NEW QUADRANT

Used as an analysis tool, allows user to place quadrant markers on a dot plot. Move the quadrant center by left clicking on the cross of the x and y bars and drag the center to the desired location. Statistics displayed at the bottom of the plot will display data statistics of each quadrant Left Top (LT), Left Bottom (LB), Right Top (RT), and Right Bottom (RB).

DELETE QUADRANT

Allows user to delete the quadrant displayed on the dot/density plot.

CONVERT TO

Allows the user to toggle the plot display between a density plot or a color dot plot. Check the desired plot display type and click OK. Regions and statistics are unaffected.

DELETE PLOT

Removes the dot/density plot from the sample template.

NOTE: that any gating regions in the plot will be removed from the gating hierarchy. This may result in deleting or reorganizing other regions that are dependent upon the plot that is deleted.

PRINTING

This feature allows the user to 1) Print the data contained within the dot/density plot, 2) Look at the print set up conditions for the dot/density plot, and 3) Save the dot/density plot image as a .bmp file.

12.3 ADD PROFILE GRAPH

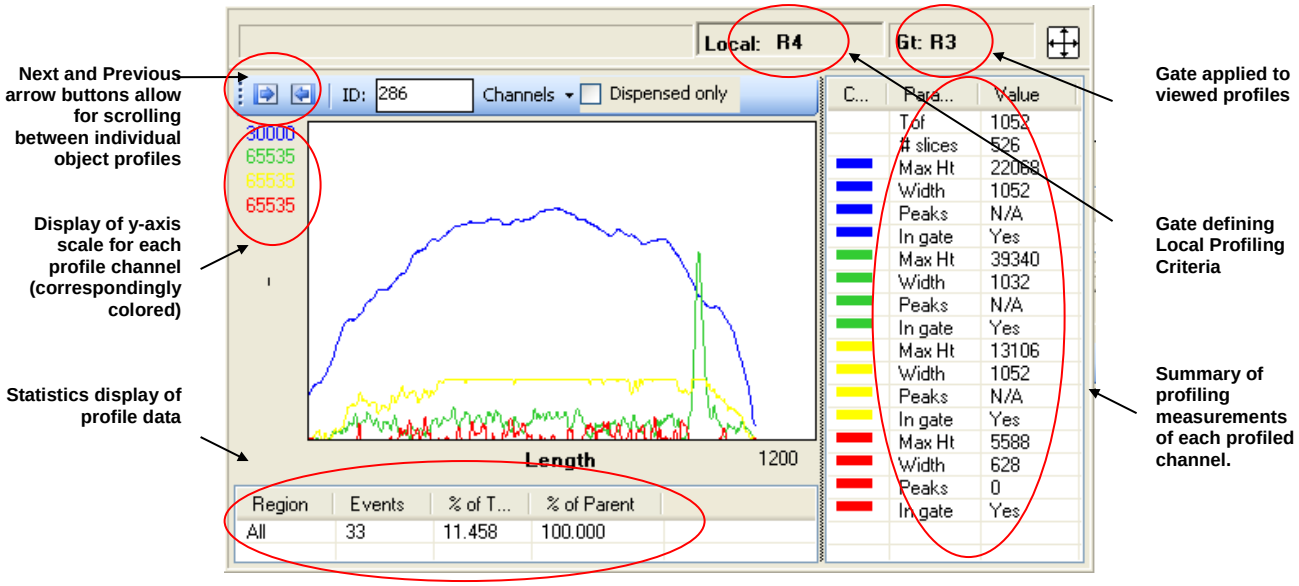


Figure 56 Profile Graph

12.4 DISPLAY OF A PROFILE GRAPH

Profiles are plotted with the object length (time of flight) along the x-axis (max value displayed at bottom right corner of the graph). The y-axis displays signal intensity changes detected for each channel as overlaying line graphs of each parameter (correspondingly colored) plotted along the object's length-scale for each profile channel is

ID: This is the profile identifying number that corresponds to its location in the txt file of the stored data. User can input an object ID number and hit enter to show the profile of that object.
NEXT/PREVIOUS arrow buttons: These activity buttons allow the user to scroll through successive profiles ordered by index number.
Dislay dispensed objects: checked this feature only plots the object profiles of objects that have been dispensed. Choose all dispensed objects or objects from certain well locations.
PARAMETER SUMMARY:
TOF: Time of flight of the current object
Max Ht: This is a numerical value of the parameter's highest peak within the currently displayed profile.
Width: This is the summation of all peaks' widths as determined at the user defined "calculate width on level" for the currently displayed profile.
Peaks: displays the # of peaks identified in the object (if this feature is active).
In Gate: displays whether the object fulfilled the profile selection criteria.

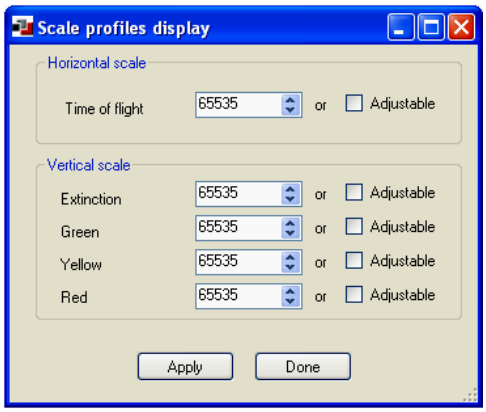
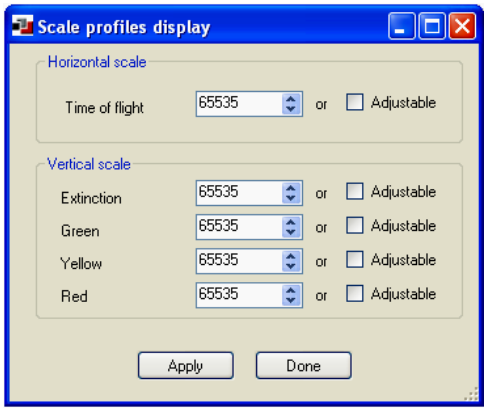


Figure 57 Rescale Profile Display

Summary of graph statistics: displays the statistics for the events acquired at the bottom of each graph. The statistic summary accounts for regions placed on the graph. The statistical summary includes the number of events to occur within a given range region, the percentage the region contains of the total events, and percentage of the parent region

12.4.1 MODIFY THE PROFILE GRAPH



SCALE

Change the horizontal or vertical scales independently. Use either the arrows to the right of each field or manually input values to change scaling.

Select 'Adjustable' to plot each parameter as it will 'best fit' within the profile window. Click APPLY to accept the changes and the DONE button to close the dialogue box.

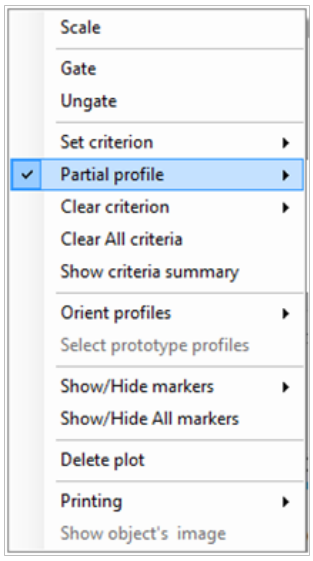


Figure 58 Profile Option Menu

GATE

Allows the user to select events (contained in another plot region) to be displayed on the profile graph. Select on the gating regions from the list provided and click OK. Note that when gating is applied, only data from the selected plot/gate are displayed on the profile graph.

UNGATE

Removes gating parameters from the profile graph. Profile graph will contain all acquired object events.

SET CRITERION

Allows user to set various peak height, width and peak gating/sorting criteria. See FEATURE for a detailed and sorting using profiing

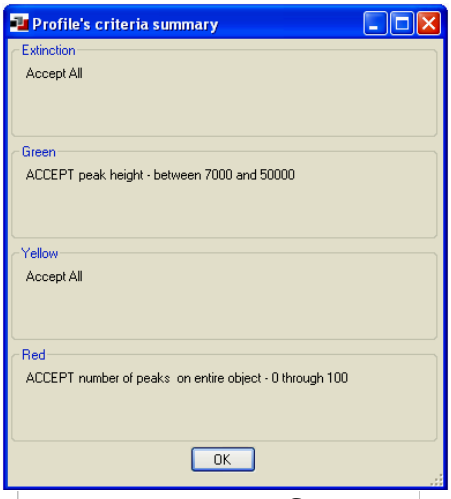


Figure 59 Profile's Criteria Summary

profiling criterion for # identification as Chapter 16 PROFILING explanation of setting criteria.

PARTIAL PROFILE

User must select the channel conditions for calculation of

in which to apply partial profiles.

CLEAR CRITERION

Allows user to deactivate individual parameter

profiling criteria.

CLEAR ALL CRITERIA

Allows user to deactivate ALL profiling criteria.

SHOW CRITERIA SUMMARY

Displays a summary of the currently active profiling criteria. (Figure 59 at right)

ORIENT PROFILES

Allows the user to set criteria to identify a head and tail of each object which the software can use to extract sample characteristics. See section 16 PROFILING FEATURE for information to properly set this feature.

SELECT PROTOTYPE PROFILES

Allows user to scroll through and select specific profiles for system to use as a prototype during profile orientation. See section 16 PROFILING FEATURE for information to properly set this feature.

SHOW/HIDE MARKERS and SHOW/HIDE ALL MARKERS

Allows user to remove some or all of the profiling markers from the display of the profile graph. These criteria are working in the background they are simply not actively displayed on the graph itself.

DELETE PLOT

Removes the profile graph from the software template. Note that any criteria selected in the profile graph is removed from the gating hierarchy.

PRINTING

This feature allows the user to 1) Print the data contained within the profiler plot, 2) Look at the print set up conditions for the profiler plot, and 3) Save the profiler plot image as a .bmp file.

12.5 ADD PROFILE STACK (TYPE I OR TYPE II)

Profile stacks can be used as an analysis tool (after sample acquisition) to line up profiles of sample objects in order to identify fluorescence peak characteristics of a sub population or the sample as a whole. In these graphs, every horizontal line is a profile with the horizontal axis displaying TOF. If there are more objects than the vertical resolution of the graph allows, then the whole image is compressed.

There are two types of profile stacks:

TYPE I PROFILE STACK:

These stacks display one parameter along each horizontal line and the intensity of the color corresponds to the value of that parameter.

TYPE II PROFILE STACK:

These stacks can display multiple parameters along each horizontal line. Unlike Type I, the color does not change intensity proportional its parameter's value, rather it only shows a certain color when that parameter is above a threshold value. You must select one parameter to be used to orient the profiles.

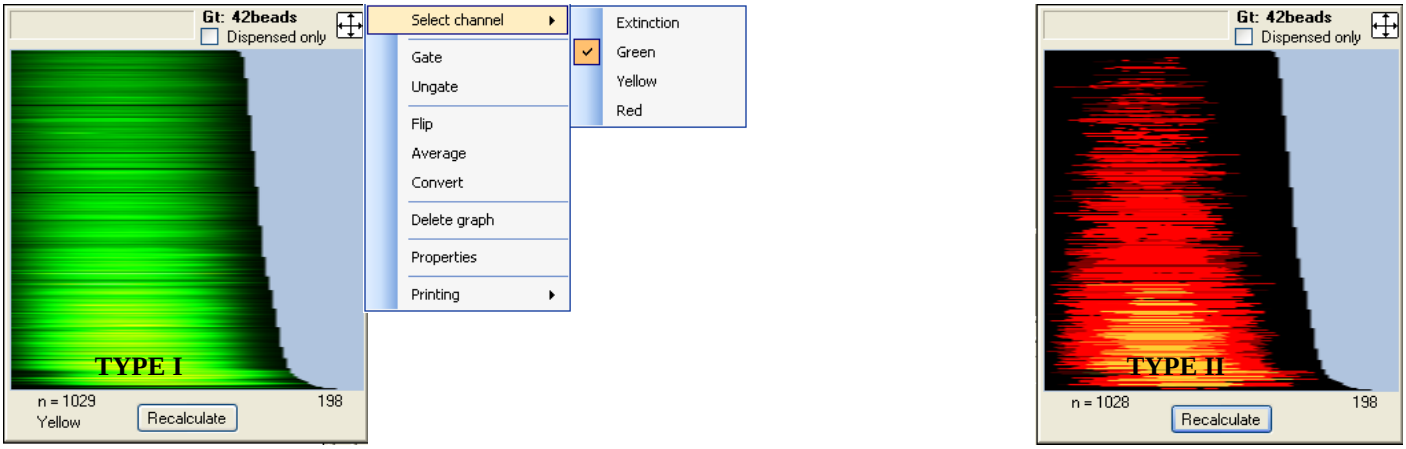


Figure 60 Profile Stack Options & Examples of Peak Height profiles stacked and sorted three different ways

12.5.1 MODIFY A PROFILE STACK

SELECT CHANNEL

Allows user to choose which profile channel to plot on profiler stack

GATE

Select a population of the sample to plot on profile stack feature.

UNGATE

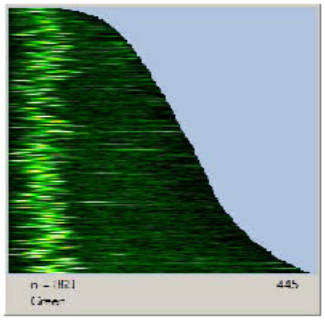
Remove gating criteria from profile stack feature. All sample events will be stacked.

FLIP

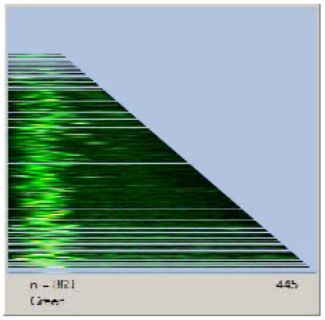
Reorient the data over the y-axis flip head to tail.

AVERAGE

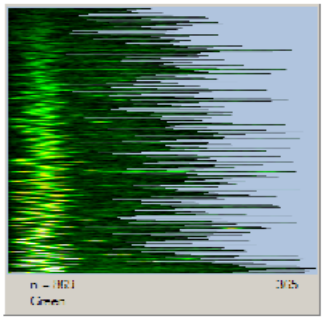
The averaging tool averages all profiles of the same length and displays them as a single line. If there are no profiles of a certain length then that line is left empty.



Display Green PH, Sort by TOF



Display Green PH, Sort by TOF w/ Averaging



Display Green PH, Sort by PH

CONVERT

Change the profile stack from type I to type II.

DELETE GRAPH

Delete the graph from the FlowPilot-Pro screen. Does NOT affect data or other displays what-so-ever.

PROPERTIES

Allows user to set properties for stacking profiles.

Minimum PCC is the Pearson Correlation Coefficient used for selecting of relevant objects and their orientation. Increase the coefficient to apply stricter rules.
Maximum intensity is the signal intensity coded with maximum color (white)
To Orient profile use channel: Match profiles on the selected channel for orientation decision (ie. head or tail alignment).
Order by: Parameter used to order objects in profile stack. The greatest values are displayed at the bottom of the graph.

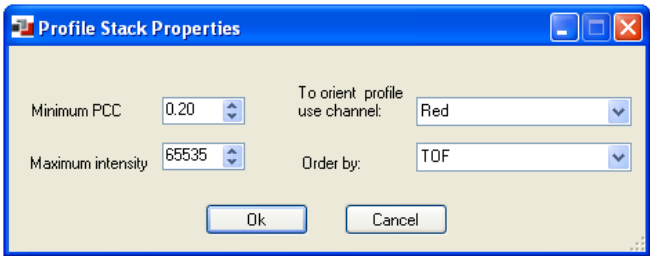


Figure 61 Profile Stack Properties

After making changes in the Profile Stack Properties dialogue box, select OK to accept changes and close the dialogue box.

PRINTING

This feature allows the user to 1) Print the data contained within the profiler plot, 2) Look at the print set up conditions for the profiler plot, and 3) Save the profiler plot image as a .bmp file.

12.6 ORGANIZE GRAPHS

Clicking this feature will organize graphs. Organized graphs are simply moved to overlay each other at the top left corner of the software screen so that the graphs overlay each other in a cascade where only the heading bar is exposed for each graph.

12.7 DELETE GRAPHS

Clicking this feature will delete all graphs that ware currently on the screen. This will effectively erase any gates and regions as well. For this region a warning message will appear asking the user if it is OK to erase all the graphs.

12.8 STATISTICS

12.8.1 CHANGE STATISTICS DISPLAY

Choose to display:
Number of total events, the % the region contains of the total events, % of the parent region, Mean X/Y, Geometric Mean X/Y, CV (coefficient of variation) of X/Y as a percentage, and/or Standard deviation of region events using X or Y values.

To use:
Select the statistics features you'd like to display by placing a check mark next to the feature.
Choose whether to Apply to all graphs or not and click DONE to close the dialogue box and return to the active FlowPilot Software screen.

If necessary, REFRESH the displayed statistics by clicking on the green arrows forming a circle on the software tool bar. OR open the Data tab from the MENU bar and choose to Refresh data display.

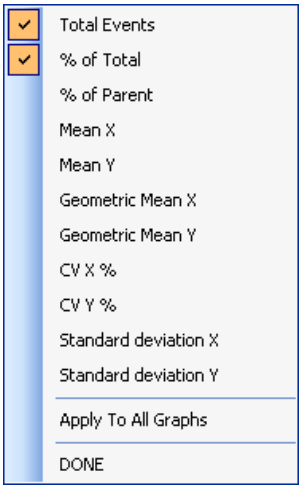


Figure 62 Profile Statistics Display

Figure 63 Profile Statistics Display

13 DATA MENU FEATURES

13.1 STORE DATA

Click on this heading to store currently acquired data. This function can also be performed using the store button on the menu bar.

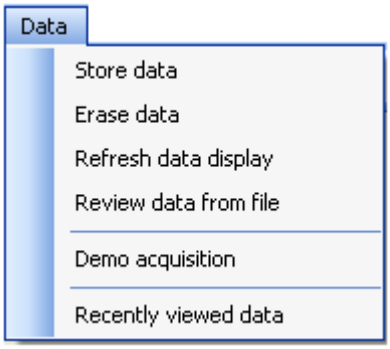


Figure 64 Data Menu

13.2 ERASE DATA

Click on this option to erase currently acquired data.

NOTE: Unless instructed to store data was initiated at the beginning of data acquisition, all the current data will be erased. This function can also be performed using the erase button on the software tools menu bar.

13.3 REFRESH DATA DISPLAY

Click on this option to refresh data including gates and statistics display.
This function can also be performed using the refresh button on the software tools menu bar.

13.4 REVIEW DATA FROM FILE

Click on this option to choose a previously acquired data set to display on the software screen. User may choose to import the sample template along with the data to be viewed OR review/plot data using the current sample template (including plots, profile windows and gates). This function can also be performed using the Review Data button on the software tools menu bar.

13.5 DEMO ACQUISITION

Click on this option to re-acquire previously stored data. Currently only available on demo model instruments.

13.6 RECENTLY VIEWED DATA

See a list of recently viewed data files. Selecting a recent data file will initiate the system to review the data file.

14 MAINTENANCE MENU FEATURES

14.1 BLEACH WASH

Clicking the bleach wash feature will start an 8 minute timer during which the 50% bleach solution is circulated from the cleaning container through the instrument (flow cell, sample cup). This feature should be followed by the WATER WASH feature a few times.

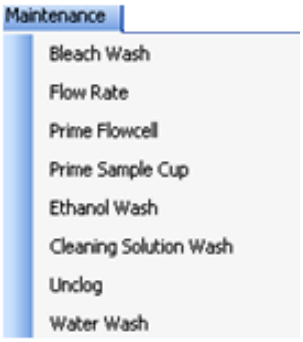


Figure 65 Maintenance Menu

14.2 FLOW RATE

Clicking the flow rate feature will start a 1 minute timer allowing the user to accurately time a 1 minute collection of sheath flow.

14.3 PRIME FLOW CELL

Clicking Prime flow cell activates sheath to flood the flow cell and pre-analysis chamber and to exit via the evacuation port on the pre-analysis chamber. This is often used to remove bubbles or debris from the pre-analysis chamber.

14.4 PRIME SAMPLE CUP

Clicking the Prime sample cup activates the system to open the Sample valve and allow sample to fill the tubing for x seconds. This should be done with a filled sample cup in place.

14.5 CLEANING SOLUTION WASH

Clicking the cleaning solution wash feature will start an 8 minute timer during which the cleaning solution is circulated from the cleaning container through the instrument. This feature should be followed by the WATER WASH feature a few times.

14.6 UNCLOG

Clicking the UNCLOG cycle depressurizes the sample cup while the sheath and sample valves are open allowing sheath to move through the sample line and into the sample cup.

14.7 ETHANOL WASH

Clicking the ethanol wash feature will start an 8 minute timer during which the 70% ethanol solution is circulated from the cleaning container through the instrument. This feature should be followed by the WATER WASH feature a few times.

14.8 WATER WASH

Clicking the cleaning solution wash feature will start a 5 minute timer during which the water is circulated from the water container through the instrument.

15 ABOUT

15.1 REGISTRATION

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

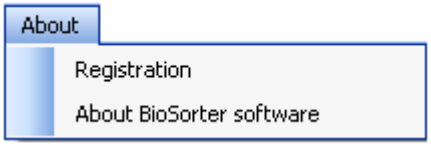


Figure 66 About Menu

15.2 ABOUT BIOSORTER

Displays the software and firmware revision numbers.

16 PROFILING FEATURE

16.1 PROFILER: AN OVERVIEW

The standard BioSorter system measures the value of extinction and fluorescence signals by integrating each signal over the time that the threshold signal is above the threshold value. The result for each signal is a single value that has obscured any details of the changing intensity of the signal while the signal is being integrated. Therefore, an object containing a small intense fluorescent spot and another object with a low diffuse level of florescence throughout the object would appear the same despite dramatically different spatial organization of the fluorescence signal.

Instead of making a single integrated measurement of a signal, the Profiler option digitizes the instantaneous signal level. The result is a list of successive point measurements made while the object passes through the flow cell. An object containing a small bright fluorescent spot will produce a fluorescence signal with a corresponding narrow peak, and the Profiler will digitize the peak into a succession of numbers that directly trace the fluorescence peak as it passed through the flow cell.

16.1.1 POSITIONAL INFORMATION

An additional advantage of the Profiler is that all digitized points have been recorded proximally, along with the peak signal, so that the position of the peak can be located proportionally in the total list of points. This permits extracting positional information from the complete profile, rather than simply the presence or absence of peaks. Notice how the fluorescence profile signal aligns with the microphotograph in the figure below. Profiles can be collected for the changes in optical density (which we refer to as extinction or EXT) and three fluorescence channels, along the length of the object, simultaneously. This correspondence permits testing for the relative positions of fluorescent markers within an object, allowing another dimension for resolving differences between the analyzed individuals in a collection or population of objects.

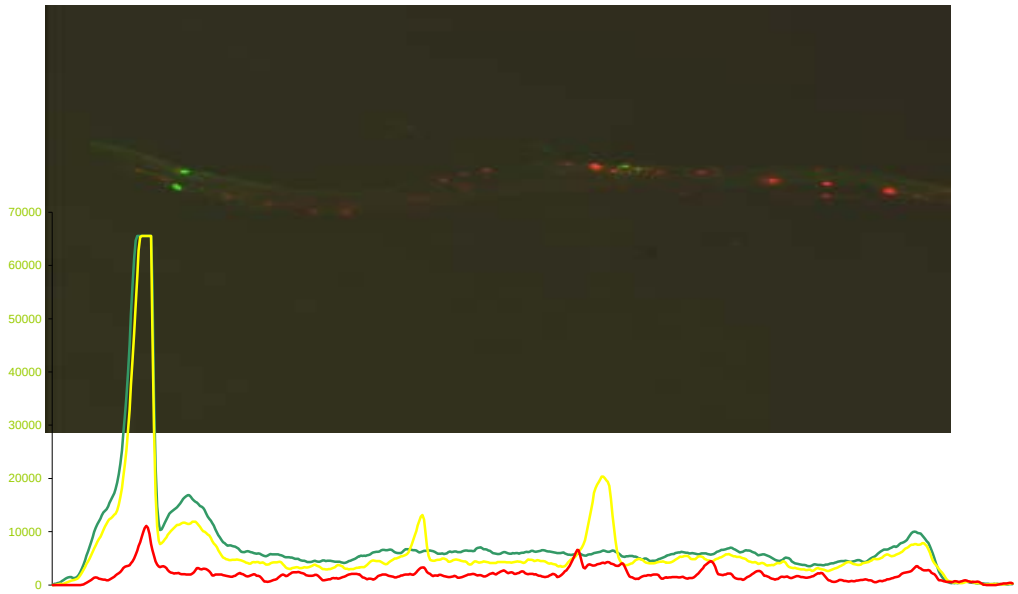


Figure 67 Profiler Image

C. elegans worm with str-1:: GFP; mab-5::dsRed; unc-17::zsYellow
Profile of transgenic nematode clearly shows green expression in head (at left), yellow expression in the animal's vulva and red expression in multiple specific cells along the body.
Representative worm image generated from standard DIC Fluorescent microscope, profile graph does not originate from imaged worm.

16.1.2 MULTIPLE PARAMETER DATA ACQUISITION

Profiler allows for sorting based on profile features of multiple parameters as a secondary sorting mechanism for objects fulfilling sort regions in dot plot. In the absence of profiler, most settings can be manipulated so that slightly differing subpopulations are separated on the various gate and sort dot plots. However, with activation of profiler, "sortable" objects fulfilling dot plot criteria can be further analyzed for presence/absence of a peak of interest in one or more profiled parameters. This means that a sort decision can be made based on any user defined profile features contained in one or more of the profiled parameters. Moreover, specific sorting rules can be established for each profiled parameter independently, and only events that fulfill ALL the sorting criteria will be dispensed.

16.1.3 NUMBER OF PEAKS

Finally, software can count individual fluorescence peaks over the entire length of the object. Using user defined criteria, the system will determine peaks within an object then compare to count limits set by user before a sorting decision is made.

16.2 OPEN A PROFILER GRAPH

Under LAYOUT menu heading select Add Profile graph

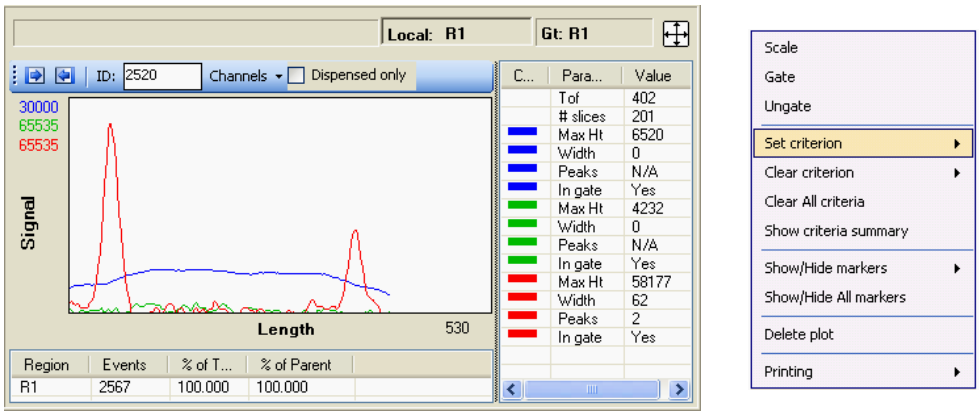


Figure 68 Adding a Profile Graph

16.3 CHANGE THE SCALE OF PROFILES

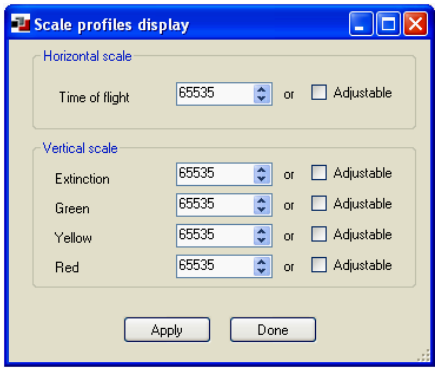


Figure 69 Rescale Profiles

The profile of each object will be displayed in the profile window so that a line graph of each parameter profiled (correspondingly colored) will be graphed along the TOF measurement (x-axis). Because each parameter's information will be unique to each sample, it is necessary to set the display scale of each parameter independently. This allows the user to adjust how each parameter is displayed in the profile window as the data is acquired.

1. Right Click in the body of the Profile graph you would like to amend. Open the Scale dialogue box under the PROFILER pull down menu.
2. Adjust the Horizontal scale (Time of Flight) to fit the objects within the profiling window. Or select adjustable to allow max scaling according to the time of flight of individual objects. Use up or down arrows or input values to adjust the scale settings.

NOTE: If profiler plots an object with a TOF larger than the maximum, the profile may appear truncated within the profile window.

3. In like fashion, adjust the Vertical scales of each profiled parameter (or select Adjustable) to plot each fluorescence signal appropriately within the profiling window.

NOTE: Keep in mind if a profiled parameter exceeds the limits set in scaling, the profile graph will contain a flattened peak at the maximum y-axis value (upper scale limit). It may be necessary to change the scaling. However, if you find that a peak exceeds system maximum value of 65536 (signal is saturating the channel's storage limits) you may consider changing scan rates, PMT, or gain settings for this parameter. See sections 8.3.1 SCAN RATE and Error! Reference source not found. **for more information on changing these variables.**

4. Select OK to implement scale settings.

NOTE: Scaling only affects how the profile is displayed, not the raw data generated. Profile scaling can be changed at any time during data acquisition.

16.4 GATE AND UNGATE PROFILE GRAPH

Choose an existing region to selectively display a subset of all data objects in the profiling window OR ungate to plot all objects.

16.5 SET CRITERION

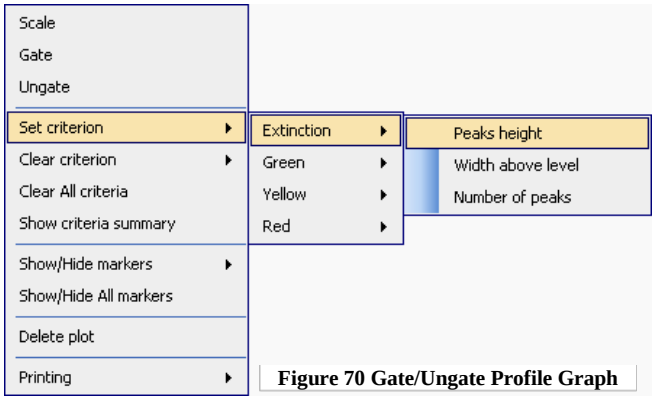


Figure 70 Gate/Ungate Profile Graph

In the profiling version of the software, Some of the parameters generated for each object are the peak intensity of the highest peak as well as the peak width (at a user selectable value) for each parameter profiled: Extinction, Green, Yellow, and Red Fluorescence. User has control to set criterion for determining peak height and width limits as well as determining number of peaks within an individual object.

NOTE: These limits then become part of the region criteria for dispensing purposes. The displayed region ie, 'local: R2' will contain all your set limits for profile determination.

16.5.1 PEAK HEIGHT LIMITS

Peak height is the highest measured value along the length of the object. Use arrows or input appropriate peak limits for High and Low markers. Then select whether to Accept All, Accept, or Reject the marked limits. Click OK to implement the criteria.

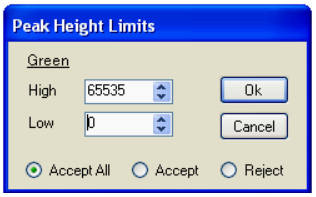


Figure 71 Peak Height Limits

Accept All

Ignoring the specified range for the 'Peak Height', every object fulfilling other gate/sort criteria will be dispensed.

Accept

Only those objects that are within the specified range for the 'Peak Height' will be dispensed.

Reject

Only those objects that are outside the specified 'Peak Height' range will be dispensed

16.5.2 WIDTH ABOVE LEVEL / TOTAL WIDTH LIMITS

The peak width is determined when the software calculates the sum of all lengths of the profile that exceed the user selected signal level (**Calculate total width above signal level**). The calculated width is then compared to user selected Low and High limits for acceptance or rejection. (Accept All, Accept, Reject).

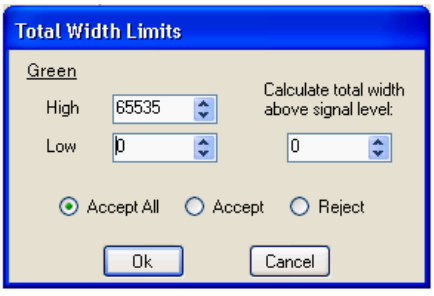


Figure 72 Total Width Limits

Accept All

Ignoring the specified range for the 'Peak Width', every object fulfilling other gate/sort criteria will be dispensed.

Accept

Only those objects that are within the specified range for the 'Peak Width' will be dispensed.

Reject

Only those objects that are outside the specified 'Peak Width' range will be dispensed.

16.5.3 NUMBER OF PEAKS

One of the most useful features of the Profiler feature is its ability to identify individual peaks (extinction and/or fluorescence) within each object.

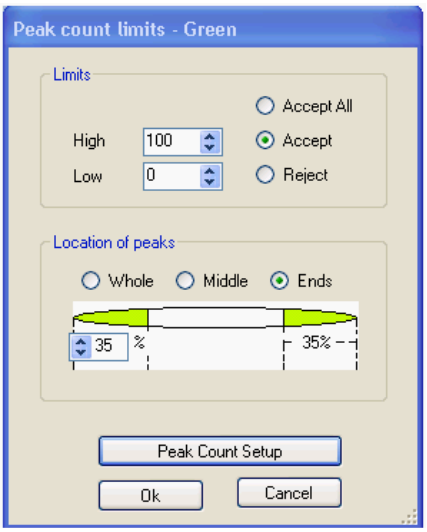


Figure 73 Peak Count Limits

LIMITS

After counting peaks, system will check the peak number limits to see whether they fall within the limits set by the user. Set Low and High limits for PEAK NUMBER And whether to Accept All, Accept, or Reject the set limits once peaks have been identified.

LOCATION OF PEAKS

Location of peaks is the relative position of peaks, expressed as a % of length of the whole object, in the profile. This allows the user to specify a segment over which the number of peaks is evaluated. Notice that user MUST set limits to evaluate peaks over the WHOLE, MIDDLE, or ENDS of the object.

NOTE: It is very important to consider the orientation of the object as it moves through the flow cell and path of the laser(s). The orientation of asymmetrical objects will more often assume a linear position as they enter the narrow sample stream, however, it is often the case that these objects can tumble or move (if they have motility) and do not appear to move through in a linear fashion. In this case identifying orientation may be more difficult, ie the beginning of the profile may not be the head of the object.

WHOLE

Ignores limits set.

Peaks along the whole profile are included in the 'Number of Peaks' count; the 'Location of peaks' limits are disregarded.

MIDDLE

Middle will check for peaks appearing WITHIN the % limits set.

Only peaks between the % marked locations are included in the 'Number of Peaks' count (effectively excludes the ends). The shaded area of the object in the dialogue box represents the location in the profile in which it will identify peaks.

ENDS

Ends will check for peaks satisfying criteria OUTSIDE the % limits set by user.

Only peaks outside the 'Location' limits are included in the 'Number of Peaks' count, i.e. only the 'heads' and 'tails' outside the middle are taken into account. The shaded area of the object in the dialogue box represents the location in the profile in which it will identify peaks.

16.5.4 PEAK COUNT SETUP

Set up to identify peaks within the object profile.

AVERAGE BY # PEAKS

The program looks at every data point of the profile, averaging data values from surrounding channels to construct a smoothed graph in which to identify peaks. This is designed to remove the little variations in fluorescence along the profile in essence reducing the fluctuations based on noise/autofluorescence.

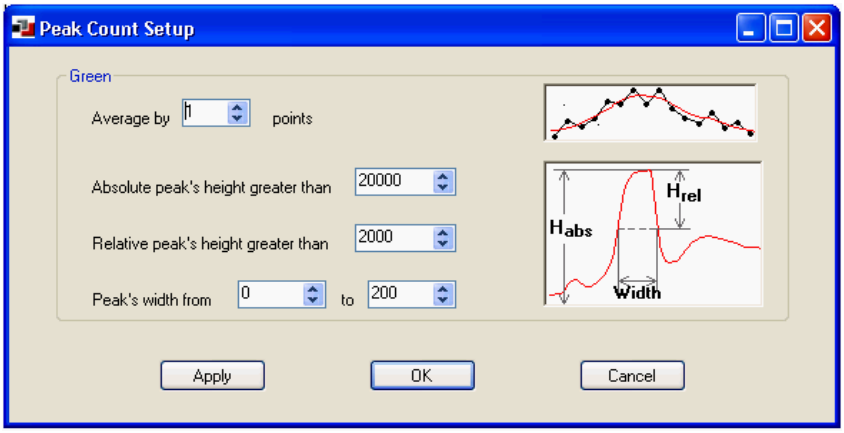


Figure 74 Peak Count Setup

NOTE: Consider the scan rate and object size.

Higher scan rates generate a data point as often as every 0.2 microseconds. The data reflects a very small slice of the total length of the object. Conversely, the slowest scan rate collects data over 6.4 microseconds as the object passes the laser path. Effectively, each data point contains information over a longer slice of the total length of the object. One way to think about this is to consider that each data channel generated using the slowest scan rate may contain information that spans up to 32 points generated using the highest scan rate. In general it is a good idea to use higher scan rates for samples where the object contains very small localized signals that may be blended using lower scan rates, whereas longer objects can almost always utilize lower scan rates.

Once the user inputs the number of channels to average on, the "smoothed" profile is drawn in black on top of the actual profile and this becomes the profile line graph that the program uses to determine and count peaks.

1. Determine a threshold value for determining a peak. This is **Absolute peak's height greater than value (H_{abs})** in the peak count dialogue box. See the discussion of peak's absolute height below. Enter an integer into the window.

ABSOLUTE Peak's height greater than (H_{abs})

The absolute height of the peak is a signal level that the graph needs to rise above in order to be analyzed as containing peaks. Keep in mind that any area of the profile that does not meet this value will be excluded from analysis as a peak, so it is important to determine a height sufficient to contain all peaks of interest.

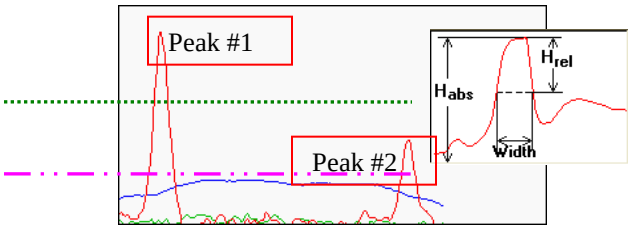


Figure 75 Peak Option Layout

Example: On the profile above, notice using a high threshold (dotted green line value ~40000) produces only a single peak(peak #1)) for count analysis while a lower threshold value (purple dashed line~20000) allows for determination of 2 peaks (peaks #1 and #2).

2. Input a Relative peak height for peak identification. **Relative Peak's height greater than (H_{rel})** The relative height will determine where each "potential" peak's width is calculated. See discussion below to determine peak's relative height greater than.

RELATIVE Peak's height greater than (H_{rel})

Once the program identifies an area of the profile that meets absolute peak height, it locates each individual peak maximum value and analyzes these points for peak relative height and peak width. At each maximum, the program moves down the peak the distance of the user-defined relative height to determine the width of the peak at this point. Basically, the program extends a line across the peak at this height (peak height – relative height). Wherever the line intersects with the graph twice it creates endpoints that it will measure to determine the width of this peak. **This distance is the single peak's width (Width)**

The value set for relative peak height effects which peaks are counted. Low and wide peaks may require different identification criteria than high and narrow peaks.

3. Set limits for **peak's width**. Set upper and lower limits for the peak width for peak analysis by entering values in the "**Peak's width from"** boxes. Determined width of peak must lie within these limits to be counted as a peak.

NOTE: a generous set of limits will favor counting many peaks while a smaller range may exclude peak widths just outside either end of the limits. It is useful to determine the best set of limits by applying different value sets and scrolling through some object profiles to identify the conditions that work best for your sample.

4. Click Apply to implement all the settings.
5. Click OK to close the dialogue box and return to viewer screen. Profile viewer will have additional items displayed. A black line (average on) graph closely laid over the parameter channel used to count peaks should appear on the display. Additionally, user should see individual peaks shaded with a light color across each peak denoting where the width measurement was made.

16.6 CLEAR CRITERION / CLEAR ALL CRITERIA

Clicking on these options allows the user to clear an individual profile criterion or clear all criteria.

16.7 SHOW CRITERIA SUMMARY

Click on this feature to show a summary of the current profile criteria for each channel.

Note: this is the selection criteria for dispensing the local profiling gate.

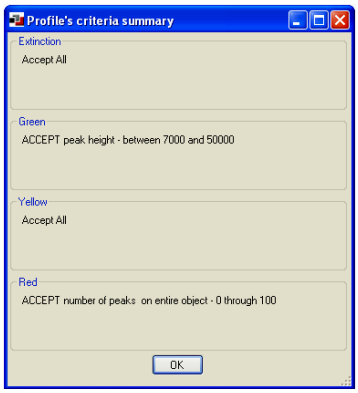


Figure 76 Profile Criteria

16.8 SHOW/HIDE MARKERS AND SHOW/HIDE ALL MARKERS

Click on this feature to show or hide an individual or all the profile criteria markers on the profile window. These include: Peak Heights limits, Peak width level of determination, and profiling peak location markers.

16.9 DELETE PLOT

Click on this feature to delete the profile window. Note: any gate region dependent on the local profiling criteria may be affected.

16.10 PRINTING

This feature allows the user to 1) Print the data contained within the profiler plot, 2) Look at the print set up conditions for the profiler plot, and 3) Save the profiler plot image as a .bmp file.

17 DATA STORAGE

Upon acquisition, the system requires the storage of the acquired data. Several data files are stored at this time: xxx.bxrt, xxx.lmd, xxx.txt

17.1 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.

17.1.1 DESCRIPTION OF COLUMNS IN THE SUMMARY DATA FILE

ID

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

TIME

This is a time stamp of when that object was detected in the course of the day's instrument use.

PLATE

Plates are indexed in chronological order from the time the instrument was last turned on. This correlates with the 'Plate #' display on the acquire/dispense dialogue box.

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.

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.

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.

STATUS SORT

A number representing the sorting status of an event, returned by *BioSorter* board.

Refer to the following key:

0-just acquiring
1- out of sort gate
2 -sortable, but too late
3 - out of sync
5- sorted in shortened drop, due to being too close to previous
6- sorted
8- same as 5 in "extra pure" mode does not get dispensed
9- coincidence with previous
10- coincidence with following
12- sorted, within sorted drop another sortable object discovered after sorting impulse has been issued
13- sorted, within sorted drop not sortable object discovered after sorting impulse has been issued

IN REGIONS

This is an identifying code indicating which gating regions/criteria the object fulfilled. This number is read from right to left and contains an integer column for each gate region contained in that data acquisition such that the integer 0 indicates the object was not in the gate region while an integer of 1 indicates the object was inside the drawn gate region.

Example: The value 1011001 contains data for 7 drawn regions and can be read as follows: from right to left: inside (1) r1, outside (0) r2, outside r3, inside r4, inside r5, outside r6, inside r7. If the value appears truncated when the txt file is opened, it is because the object stored 0's for the last gate regions: 0001101 is truncated to read 1101 in the txt file.

TOF

Time of flight value

EXT

Extinction integral value

GREEN, YELLOW, RED

Integral values for fluorescence on a corresponding channel.

PROFILING PARAMETERS

Contain features only utilized with the profiling option. See section 16 PROFILING FEATURE discussing the Profiling feature. Without profiler enabled all columns will contain 0.

PH Extinction (or Green, Yellow, Red) indicates the highest peak intensity measured within the object.
PW Extinction (or Green, Yellow, Red) indicates the calculated width of the signal at the user defined value.
PC Extinction (or Green, Yellow, Red) stores the number of peaks identified in that object, if user has set peak conditions for determining peak count.

DATA/INSTRUMENT SUMMARY

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:

How the data was stored: Auto-stored after acquisition, or manually stored after acquisition
The FOCA/flow cell size used during the experiment, Scan Rate, Trigger source, Signal threshold, and minimum time of flight.
Signal gains for each parameter, extinction detector power, active lasers and laser power, PMT voltage applied to each channel.
Pressures applied to sheath, sample cup, and DIVERTER.
Sort settings: drop width, sort delay,
Regions applied:R1, R2, R3…etc and coordinate positions of vertices of region boundaries
Sort gate: lists the gate used as dispensing criteria
Stored data gate: gate used for data storage (if a subset of the data was chosen for data storage)
Data stored: Notes condition of stored data, i.e. whether compensation or other mathematical manipulations were applied.

17.2 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.

17.3 A FILE IN BOSORTER FORMAT 'XXX.BXRT

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

18 WORK AREA AND FACILITIES REQUIREMENTS

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

18.1 WORK AREA REQUIREMENTS

The BioSorter instrument requires a permanent, open, level, vibration free, working space measuring 3 feet (92 cm) deep x 6 feet (183 cm) wide x 3 feet (92 cm) above table height for optimal computer and instrument placement. The optional Union Biometrica supplied Air Compressor requires approximately 20 inches (51 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.

18.2 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.

18.3 ELECTRICAL REQUIREMENTS

A dedicated power cord and power strip is used on this system. The power strip is to be used only with BioSorter instrument components. Do not plug anything other than BioSorter instrument components into this dedicated power strip.

The BioSorter instrument does not contain any user-serviceable electrical components. Do not remove any of the safety covers. Risk of shock due to hazardous voltages exists if the instrument is run with the safety covers removed.

CIRCUIT REQUIREMENTS FOR 120 VAC COUNTRIES

BioSorter Instrument:	100-120VAC, 15-20 Amp, 50/60 HZ, single phase with protective earth ground.
Air Compressor (optional):	100-120VAC, 15-20 Amp, 50/60 HZ, single phase with protective earth ground.

CIRCUIT REQUIREMENTS FOR 230 VAC COUNTRIES

BioSorter Instrument:	220/240VAC, 10-16 Amp, 50/60 HZ, with protective earth ground.
Air Compressor (optional):	220/240VAC, 10-16 Amp, 50/60 HZ, with protective earth ground.

FUSE REQUIREMENTS FOR THE BIOSORTER INSTRUMENT

BioSorter Instrument line fuses should not be substituted. Replace with Union Biometrica parts only.

Specification:	5.0 A / 250V, 5 x 20 mm, Slow-Blow Fuse. Approved to: IEC 60127-2
Union Biometrica part number:	067-0002-015 (two required for 230 VAC countries)

18.4 AIR REQUIREMENTS

The input air pressure should be at least 40 PSI [2.7 bar] but no more than 100 PSI [6.9 bar] of filtered, at 2 CFM [60 lpm], of non-condensing, water and oil free air. The optional Air Compressor is available for laboratories that cannot meet these requirements.

CAUTION: Main input regulator must be set to 40 PSI [2.7 bar].

19 REAGENTS

Performance Specifications are only valid with Union Biometrica reagents. DO NOT USE UNAPPROVED SOLUTIONS in the BioSorter instrument. 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.

19.1 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. For most BioSorter applications, *COPAS* GP Sheath is recommended.

370-5070-000 cell sheath reagent is recommended for use with all live organisms and cellular based applications. GP sheath 300-5070-100 can be used for c. Elegans and bead based applications.

19.2 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 BioSorter applications, GP 42 Micron Control Particles are recommended for use.

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

19.3 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.

19.4 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 BioSorter applications.

19.5 STERILIZATION SOLUTION

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

19.6 BLEACH

Bleach is used on the BioSorter 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™ 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).

20 SPECIFICATIONS

20.1 BIOSORTER INSTRUMENT GENERAL SPECIFICATIONS AND LIMITATIONS

Table 2 Instrument Weight

Instrument weight	Main Instrument: 70 Kg (150 lbs) Fluidics Caddy: 10 Kg (20 lbs)

For more details, please refer to section 18 WORK AREA AND FACILITIES REQUIREMENTS

20.2 GENERAL PERFORMANCE SPECIFICATIONS

20.2.1 ANALYSIS AND COUNTING RATE

Up to 1000 events per second but this number is sample and FOCA dependant. We recommend running slower to avoid the possibility of clogs.

20.2.2 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.

20.2.3 AUTOMATED DISPENSING ACCURACY AND PRECISION

of wells with 1 bead :
≥ 97.5% accuracy ≤ 2 coincidental events per 96 well multiwell plate.

# of wells with >1 bead:
≥97.5% accuracy ≤ 3 coincidental events per 24 well multiwell plate

20.3 FLUID MECHANICAL DESIGN SPECIFICATIONS

20.3.1 SAMPLE CAPACITY

Sample cups are a swappable part. Choices available include: 1 Liter, 2 Liter, and 50 ml conical tube.

20.3.2 SAMPLE CONCENTRATION

Typically 1-2 objects per microliter, however this number is adjustable.

20.3.3 SAMPLE FLOW RATE

Adjustable, standard set by running controls beads at ~10-30 events/second (from a 50ml conical).

20.3.4 SAMPLE MIXING

A sample/mixing probe dips into the sample cup and spins at a user adjustable rate. There may be a few mixing paddle attachments available to customize the sample/mixer probe.

20.3.5 SHEATH FLOW RATE

Specific to individual flow cells: 250 um requires sheath flow rate of 9-10 ml per minute,
500 um flow cell requires 25 ml/min flow rate, 1000 um flow cell requires 40-45 ml/min flow rate, 2000 um flow cell requires 53-57 ml/min flow rate. These conditions are set at the factory and at installation but are adjustable by user if necessary.

20.3.6 CLEANOUT BOTTLE CAPACITY

Cleanout Bottle maximum capacity is 9 liters.

20.3.7 SHEATH BOTTLE CAPACITY

Sheath Bottle maximum capacity is 9 liters.

20.3.8 DIVERTER MECHANISM

Air jet fluid switch activated from signal processing electronics.

20.4 OPTICAL ASSEMBLY DESIGN SPECIFICATIONS

20.4.1 LASER

Up to 4 solid state lasers may be installed. A 488 nm laser operating at up to 50 mW of power is standard. Additionally, any 3 of the following lasers are available and other can be special ordered:

Table 3 Laser Wavelength and Maximum Power

Laser Wavelength	Maximum Laser Power
405 nm	100 mW
445 nm	40 mW
458 nm	75 mW
514 nm	100 mW
532 nm	100 mW
561 nm	100 mW
640 nm	100 mW
660 nm	100 mW
785 nm	40 mW

20.4.2 LASER OPTICS WITHIN THE FLUIDICS AND OPTICS CORE ASSEMBLY (FOCA)

Up to 4 laser beams converge at the plane of the flow cell.

20.4.3 FLOW CELL WITHIN THE FOCA

A quartz Flow cell lies within the Fluidics and Optics Core Assembly. Each has a square cross-section inner bore of a specific size: 250 µm, 500 µm, 1000 µm, 2000 µm.

20.4.4 DETECTORS

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

20.4.5 FILTERS

Manual and software selectable fluorescence excitation filters:
At extinction detector: 488bp, 561bp as well as several neutral density filters may be used.
At the PMT, various emission filters may be used to customize the instrument configuration for a specific set of conditions. Standard emission filter options include filters centered at: 460 nm, 510 nm, 543 nm, 585 nm, 615 nm, or 680 nm. Other filter options may be available as well.

20.4.6 AMBIENT LIGHT

Optical assembly is sealed and unaffected by normal room light.

20.5 ELECTRONICS SPECIFICATIONS

20.5.1 PROCESSORS

Several microprocessors controlling X-Y-Z Stage motion, sorting/acquisition, and fluidic control valves are utilized by the system.

20.5.2 COMPUTER

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

20.6 INSTRUMENT SETTINGS SPECIFICATIONS

NOTE: These specifications can vary depending on what the user is trying to acquire from the sample. These specifications were determined utilizing a 42 micron control particles for the 250-1000 µm FOCA and 500 micron control particles for the 2000 µm FOCA.

20.6.1 PRESSURE

Table 4 FOCA Specific Pressures

FOCA	Diverter Pressure (psi)	Sample Cup Pressure(psi)
FS	7.00-10 PSI	4.50 -5.50 PSI
250 µm	7.00-10 PSI	4.50 -5.50 PSI
500 µm	7.00-10 PSI	1.50 -2.50 PSI
1000 µm	7.00-10 PSI	0.20 -0.30 PSI
2000 µm	7.00-10 PSI	0.01 -0.05 PSI

20.6.2 GAINS

Table 5 FOCA Specific Gain Values

FOCA	EXT	FLU1	FLU2	FLU
250 µm	1.5	2.0	2.0	2.0
500 µm	1.5	2.0	2.0	2.0
1000µm	1.5	2.0	2.0	2.0
2000µm	1.5	2.0	2.0	2.0

20.6.3 PMT

Table 6 FOCA Specific PMT Values

FOCA	GREEN	YELLOW	RED
250 µm	400	450	700
500 µm	400	450	700
1000 µm	400	450	700

2000 µm	300	350	500

20.6.4 DROP

Table 7 FOCA Specific Drop Parameters

FOCA	DELAY	WIDTH
250 µm	10 ± 5 ms	6.0 ± 5 ms
500 µm	10 ± 5 ms	10 ± 5 ms
1000 µm	29 ± 5 ms	15 ± 5 ms
2000 µm	60 ± 5 ms	30 ± 5 ms

20.6.5 THRESHOLD AND TOF

Table 8 FOCA Specific Threshold and TOF

FOCA	THRESHOLD	TOF
250 µm	500	60
500 µm	500	60
1000 µm	500	60
2000 µm	500	100

21 TROUBLESHOOTING

The following categories contain the most common performance problems encountered during operation of the BioSorter instrument. Each category contains common symptoms and the basic steps recommended for assessing and correcting the problem.
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 ANY OF THE LASERS. IMPROPER SAFETY PRECAUTIONS MAY CAUSE BLINDNESS

Category 1: Pressure

Category 2: Sample Flow

Category 2: Analysis

Category 2: Sorting

Category 2: Instrument

21.1 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 that the sample pressure button is clicked or press done Refill sample to supply pressure to the sample cup/conical.
3. Check the seal on the sample cup/conical. Ensure air is not escaping from around the 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 tubing. Verify that they are properly attached to the sample cup.

Table 9 FOCA Specific Tubing and Valve

Flow Cell	Valve Connection	Inner Diameter and Fitting Colors	Part Number
250 µm	FOCA to Rotary Valve	0.5mm (Tan and White)	600-5042-005
250 µm	Rotary Valve to Sample Cup	0.5mm (White and red)	600-5042-001
500 µm	FOCA to Rotary Valve	1.0mm (Tan and Black)	600-5042-006
500 or 1000 µm	Rotary Valve to Sample Cup	1.0mm (Black and Red)	600-5042-007
1000 µm	FOCA to Rotary Valve	1.0mm (Black and Red)	600-5042-009
1000 or 2000 µm	FOCA through Pinch valve to Sample Cup	2.0mm (Red and Red)	111-0093-003

5. Adjust the sheath flow rate located on INSTRUMENT SETTINGS if necessary.

6. Take a 1minute sheath volume measurement to ensure proper sheath flow rate described in section 5.1 PREPARATION. See table below with proper sheath flow rates for specific FOCA.

Table 10 Sheath Volume Measurement

FOCA	Sheath Volume Measurement
250 µm	9-10 mL per minute
500 µm	25 mL per minute
1000µm	40-45 mL per minute
2000µm	53-57 mL per minute

7. Adjust the sample pressure if necessary see section 20.6 INSTRUMENT SETTINGS SPECIFICATIONS for details.

21.2 SAMPLE FLOW

If there is no sample flow:

1. Check sheath and sample containers. Verify they are filled appropriately.
2. Check sheath pump 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 UNCLOG button several times.
    b. Temporarily increase the speed of the sheath pump then return it back to normal operating speed. This may remove a small clog from the flow cell.
    c. Unclick SAMPLE ON and click the SHEATH ON option on the ACQUIRE/DISPENSE menu several times, opening and closing the valve. Often these steps are effective in dislodging the clog and re-starting the flow.
    d. Toggle the SAMPLE on open and closed several times. This may dislodge a minor clog.
    e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris.
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.

7. Make sure 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 correct 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 UNCLOG button 2 – 3 times.

13. It may be necessary to slightly elevate sample pressure. See table below for sample pressure increments to increase by for a specific FOCA. Increase by these increments until flow is resumed.

Table 11 Increasing Sample Pressure Increments

FOCA	SAMPLE PRESSURE INCREMENTS
250 µm	1.0 PSI
500 µm	0.50 PSI
1000µm	0.20 PSI
2000µm	0.01 PSI

If there is spray from the flow stream

1. Check sheath and sample containers. Verify they are filled appropriately.
2. Check sheath pump speed 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 in the correct position. Small adjustments may need to be made in order to bring it into correct alignment to divert the waste while allowing well formed drop during sample acquisition and dispensing respectively.
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 carefully.
    b. Soak up excess liquid around the nozzle with the tip of a paper tissue.
    c. Return the waste tray to position, click the waste tray valve several times to insure it is moving properly. While in dispensing position, observe if spray is still present below the nozzle.
6. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the UNCLOG button several times.
    b. Temporarily increase the sheath pump speed to more forcefully push sheath through the system. Then return the sheath pump speed to normal operating speed.
    c. Unclick SAMPLE ON and click SHEATH ON several times, opening and closing the valve. Often these steps are effective in dislodging the clog and re-starting the flow.
    d. Toggle the SAMPLE ON open and closed several times. This may dislodge a minor clog.
    e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris.
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.

    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 2mm 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 UNCLOG button 2 – 3 times.

21.3 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 UNCLOG button several times.
   b. Temporarily increase the sheath pump speed then return it to normal operating speed.
   c. Unclick SAMPLE ON and click SHEATH ON several times, opening and closing the valve.
   d. Toggle the SAMPLE ON open and closed several times. This may dislodge a minor clog.
   e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris.
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.

   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 2mm 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 UNCLOG button 2 – 3 times.
4. Clear the flow cell by performing the Cleaning Solution Procedure described in section 6.1 DAILY MAINTENANCE PROCEDURE.
5. Clean the interior fluidic path using the COMPLETE STERILIZATION in section 6.3 COMPLETE STERILIZATION PROCEDURE

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

1. Verify Threshold and Gains. Refer to Section INSTRUMENT SETTINGS
2. Check sample preparation for purity.
3. Check fluid levels in all bottles.
4. Check all fluid lines for leaks.
5. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris
6. Clear the flow cell by performing the Cleaning Solution Procedure described in section DAILY MAINTENANCE PROCEDURE.
7. Clean the interior optical path using the COMPLETE STERILIZATION described in section 6.3 COMPLETE STERILIZATION PROCEDURE

If sample distribution is too broad:

1. Clean the interior optical path using the COMPLETE STERILIZATION described in section 6.3 COMPLETE STERILIZATION PROCEDURE

21.4 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 FlowPilot software icon.

If the droplets do not fall within the wells:

1. Verify stage alignment according to section **Error! Reference source not found.**.
2. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the UNCLOG button several times.
    b. Place a finger over the vent on the sheath bottle cap in order to increase the sheath pressure.
    c. Unclick SAMPLE ON and click SHEATH ON several times, opening and closing the valve.
    d. Toggle the SAMPLE ON open and closed several times to gently remove a minor clog.
    e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris.
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.

    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.
4. Select the UNCLOG button 2 – 3 times.
5. 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 or perform a sort delay setup to find the optimal delay conditions for the objects.
2. Check the flow cell for a clog. Clear the flow cell by performing the following:
    a. Click the UNCLOG button several times.
    b. Place a finger over the vent on the sheath bottle cap.
    c. Unclick SAMPLE ON and click SHEATH ON several times, opening and closing the valve.
    d. Toggle the SAMPLE ON open and closed several times to gently remove a minor clog.
    e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris.
    f. 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.

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 2mm 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 UNCLOG button 2 – 3 times.
5. Clean the interior optical path using the COMPLETE STERILIZATION described in section 6.3 COMPLETE STERILIZATION PROCEDURE.

If there are multiple objects in wells filled:

1. Verify the COINCIDENCE Method reads Pure.
2. Adjust the SORT WIDTH using the field provided. Refer to section **Error! Reference source not found.**.
3. Adjust the SORT DELAY using the field provided. Refer section **Error! Reference source not found.**.
4. Check the sample preparation. Verify the concentration of the sample is within specification.
5. Adjust the THRESHOLD. Refer to section**Error! Reference source not found.**.

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

a. Click the UNCLOG button several times.

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

c. Unclick SAMPLE ON and click SHEATH ON several times, opening and closing the valve.

d. Toggle the SAMPLE ON open and closed several times to gently remove a minor clog.

e. Initiate a prime flow cell sequence (maintenance menu item) to clear the pre-analysis chamber and flow cell of any bubbles or debris

f. 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.

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 2mm 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 UNCLOG button 2 – 3 times.
9. Clean the interior optical path using the COMPLETE STERILIZATION described in section 6.3 COMPLETE STERILIZATION PROCEDURE.

21.5 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. Press 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 18.3 ELECTRICAL SAFETY & 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. Ensure that the waste stream is correctly diverted to towards the far left edge of the waste tray.
2. Check that the sample recovery jar is intact and tightly sealed to its housing.
3. Replace the waste pump tubing on the external fluids caddy according to section 6.4 REPLACING THE WASTE PUMP TUBING.

If there is intermittent pooling or drops are sometimes dispensed between wells.

1. Check for waste tray misalignment.
2. Check the angle of the diverted sheath stream. Increase diverter pressure if necessary.
3. Check for diverter malfunction.
    a. Open waste tray and turn diverter off. Repeat several times looking for errors.
    b. Attach a small bit of air tubing to the diverter nozzle.
    c. Insert the other end in a water turn repeat diverter function test.
    d. If bubbles are produced when the diverter is turned off, contact your service technician.

For any other questions or problems call:

U S – G l o b a l H e a d q u a r t e r s
Union Biometrica, Inc.
Tel: 508-893-3115
Fax: 508-893-8044
Email: sales@unionbio.com

E u r o p e
Union Biometrica – Belgium Office
Tel: +32 (0)14 570 628
Fax: +32 (0) 14 570 629
Email: sales@unionbio.com

22 APPENDICES

22.1 APPENDIX A: DAILY PERFORMANCE LOG SHEET

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

22.2 APPENDIX B: LOG SHEETS

DAILY

Cleaning Reagent Lot No.	Date	Time	Performed By

SHORT/LONG TERM SHUTDOWN

Deionized Water	Date	Time	Performed By

22.3 APPENDIX C: STERILIZATION LOG SHEET

Ethanol Solution Lot No.	Date	Time	Performed By

22.4 APPENDIX D: MAINTENANCE LOG SHEET

Maintenance Performed	Date Performed	Performed By

22.5 APPENDIX E: TROUBLESHOOTING LOG SHEET

Date	Symptom	Troubleshooting Performed	Initials

22.6 APPENDIX F: CONSUMABLES RE-ORDER FORM

CONSUMABLES RE-ORDER FORM
FOR COPAS™ & BioSorter® INSTRUMENTS
Last revision: July 2014

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

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

Qty	Part Number	Description	
____	300-5070-100	COPAS GP (General Purpose) Sheath Concentrate (40mL volume, diluted makes 10L)	
____	310-5071-001	GP 42 micron Control Particles Concentrate (250mL makes 1L)	
____	300-5072-000	COPAS Cleaning Reagent, All Platforms (1L bottle)	
____	370-5070-100	COPAS Z Sheath Concentrate (2x1L bottles, diluted makes 100L)	
____	370-5071-000	Z 500 micron Control Particles (1L bottle)	
____	300-5100-000	COPAS Cell Sheath Concentrate (1L volume, diluted makes 10L)	
____	300-5101-000	COPAS Worm Sheath Concentrate (1L volume, diluted makes 10L)	
____	300-5102-000	C/W 42 micron Control Particles (1L bottle)	**Contact**
____	360-5072-000	50,100,200,300,400,500um - Six Bead Mixed Control Particles, NIST Traceable beads, 1 Liter	**Union**
____	360-5072-100	50,100,200,300um - Four Bead Mixed Control Particles, NIST Traceable beads, 1 Liter	**Biometrica**
____	340-5014-000	ReFLx Filter 10 pack	**For**
____	111-0125-001	Waste Pump Tubing for older COPAS Biosorts & Selects, 1/8" ID x 1/4" OD (sold by the foot)	**Current**
____	112-0250-001	Waste Pump Tubing for COPAS, 1/4" ID x 3/8" OD (sold by the foot)	**Pricing**
____	111-1301-000	Waste Pump Tubing for BioSorter & XL instruments, small tubing, (sold by the foot)	
____	111-1302-000	Waste Pump Tubing for XL instruments, large tubing, (sold by the foot)	
____	111-1303-000	Waste Pump Tubing for BioSorter instruments, large tubing, (sold by the foot)	
____	070-1311-001	Peristaltic Tubing Kit for BioSorter small solution bottles, assembly with fittings	
____	070-1311-002	Peristaltic Tubing Kit for LP Sampler, assembly with fittings	
____	350-5031-000	Sterility Filter Assembly for COPAS Sheath Bottle with tubing and fittings	
____	350-5032-000	Sterility Filter Assembly for COPAS Cleanout / ReFLx Bottle with tubing and fittings	
____	600-5031-100	Sterility Filter Assembly for BioSorter Sheath Bottle with tubing and fittings	
____	350-5033-000	Air Line HEPA Filter Assembly for COPAS with tubing and fittings	
____	600-5031-200	Air Line HEPA Filter Assembly for BioSorter with tubing and fittings	

There is a $250 minimum order total. 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): _____

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