inFlux® Cell Sorter Operator Manual

Version 2.2

CAUTION

Investigational Device Limited
By Federal (Or United States) Law
To Investigational Use

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Preface

Scope

This manual concerns solely the use of Cytopeia's inFlux® high speed cell sorter (referred to as "inFlux" or the "instrument" in this manual).

Disclaimer

Cytopeia reserves the right to change its products and services at any time to incorporate the latest technological developments. This manual is subject to change without notice.

The information in this manual is provided to the customer as is, without any representation regarding its accuracy or completeness. Any reliance on or use of information contained herein is undertaken at the sole risk of the customer.

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Chapter 1 - Introduction

Summary: *This chapter provides an overview of this manual and the use of the manual in conjunction with inFlux operation, and also describes conventions used in this manual.*

1.1 Intended Use

1.1.1 *Investigational device caution*

CAUTION – INVESTIGATIONAL DEVICE *LIMITED BY FEDERAL (OR UNITED STATES) LAW* *TO INVESTIGATIONAL USE*

The inFlux is intended for use in laboratory research. Any investigational use of the device requires approval of appropriate authorities, such as the FDA. Approval for use beyond laboratory research is the responsibility of the study sponsor, not Cytopeia.

The inFlux should only be operated by properly trained personnel in accordance with this manual and site-specific SOPs developed and approved by the study sponsor. All devices, such as lasers, used in conjunction with the instrument must be used as indicated by the original manufacturer.

1.1.2 *Spigot software*

Spigot is Cytopeia's proprietary Windows-based software application that serves as the user's interface with the inFlux. Spigot is the only software that should be used in conjunction with the inFlux. Cytopeia is not responsible for operation of the instrument with any software interface other than Spigot.

Running additional software applications while running Spigot is not recommended (with the exception of Flowjo).

1.2 About this Manual

This manual is a resource for inFlux operators, and is intended as an introduction to the instrument and its basic operation for new operators, as well as a reference for continued use by experienced operators.

The manual contains a basic description of the inFlux, critical safety information, operating procedures, Spigot interface reference, and maintenance information.

1.3 Using this Manual

Operators of inFlux are advised to read through this manual in its entirety prior to using the instrument. For maximum benefit, operators should be familiar with the various conventions used in this manual (e.g. safety cautions and text styles), which are outlined later in this chapter.

Each chapter begins with a brief summary of the information contained in the chapter, and then presents information from general to specific.

1.4 Chapter Descriptions

This manual has six chapters:

- **Introduction** covers the instrument's intended use, describes the scope of the manual and its organization, and outlines various convention used.
- **Instrument Overview** describes briefly how the inFlux works, and provides both pictorial and textual descriptions of the main functional subsystems. This chapter will be most helpful in learning to use Cytopeia nomenclature for subsystems, components and parts.
- **Safety** contains critical safety information, including a list of potential hazards and descriptions of safety features and warning labels.
- **Operating Procedures** contains step by step instructions for operating the inFlux.
- **Spigot Interface** expands on the Spigot application guidance found in the preceding chapter, and provides a reference for advanced use of Spigot.
- **Maintenance** details cleaning and periodic maintenance and inspection information.

1.5 Document Conventions

1.5.1 *Alert Statements*

This document uses the following conventions for alerting inFlux operators to critical safety and other important information:

DANGER	**DANGER** refers to a hazard that, if not avoided, will result in death or serious injury.
WARNING	**WARNING** refers to a hazard that, if not avoided, might result in death or serious injury.
CAUTION	***CAUTION*** refers to a hazard that, if not avoided,

	might result in minor or moderate injury or in damage to the workstation.

NOTICE	**NOTICE** does not refer to a hazard, but is used to indicate important information.

1.5.2 *Instrument Description Conventions*

This manual uses the following print type conventions when describing the inFlux and Spigot:

Text Style	Meaning	Example
ALL CAPS	Instrument control, switch or knob	PLATES
ITALICS CAPS	Instrument display	*RECALL 1*
Italics	Emphasis	*Note:*
ALL CAPS SHADED	Spigot menu button or command	FILE: PRINT LEFT
Italics shaded	Spigot function button, drop down list or checkbox selection	*Sort!*

In addition, the following conventions are used when describing adjustment of the inFlux:

Stage Adjustment Axes:
The inFlux employs adjustable platforms called “stages” for focusing and aiming both illumination laser light and light emitted as a result of fluorescence or scatter (discussed in more detail later in this manual). Each stage is adjustable in the three Cartesian axes: x, y and z. The axes are defined with respect to either the illuminating light path or the path of emitted fluorescence or scatter, as follows:

X-Axis Moving the stage parallel to the light path (i.e. either to focus the illumination beam or focus the fluorescent/scattered light spot).

Y-Axis Sweeping the stage horizontally (and perpendicular to

the light path).

Z-Axis Moving the stage vertically (and perpendicular to the light path).

Nozzle Adjustment Angles: In addition to the three nozzle stage movements, the Influx nozzle assembly allows two further adjustments: Theta (Θ) and Phi (Φ) angles, defined (as viewed from the front of the instrument) as follows:

Θ Theta angle refers to a side to side rotation of the stream about the nozzle tip.

Φ Phi angle refers to an in/out rotation of the stream about the nozzle tip.

Well Deposition Unit Axes: With respect to movement of the Well Deposition Unit (WDU) collection tray, X-axis is a “right-left” movement, while Y-axis is an “in-out” movement.

[End of Chapter]

Chapter 2 - Instrument Overview

Summary: *This chapter provides the user with a functional description of the inFlux, a pictorial tour of the instrument and its main subsystems, and a brief description of each functional subsystem.*

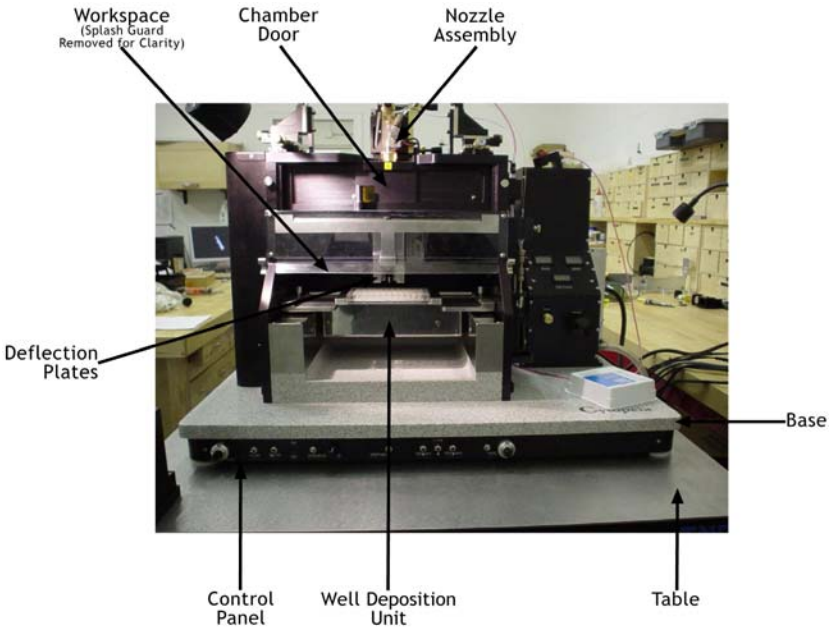
2.1 Functional Description

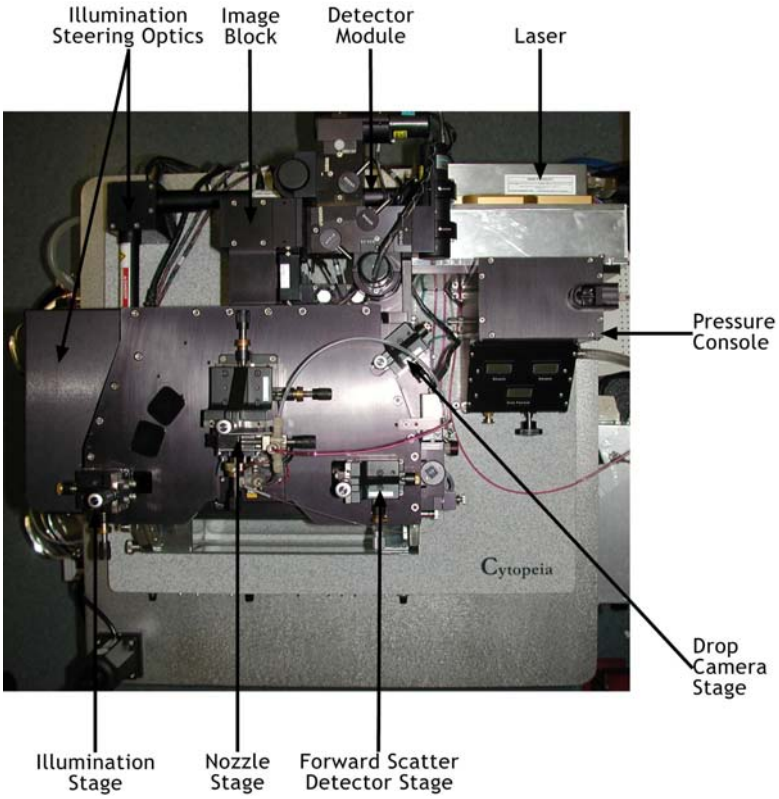
The inFlux high speed cell sorter is a research instrument that brings several technologies together to provide high-speed detection, analysis, and sorting of particles excited by laser light. Each one of these technologies, discussed in further detail below as a functional subsystem, is critical to successful cell sorting.

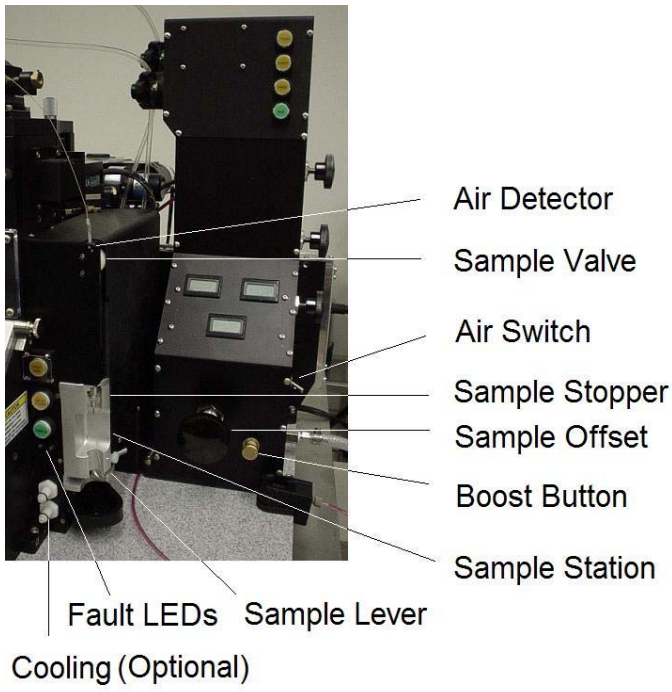
The inFlux focuses laser light on a fast-moving, thin stream of particles (e.g. cells, chromosomes, organisms) and observes the fluorescence and light scatter emitted by these particles using sensitive photomultiplier tubes (PMTs). High-speed electronics capture these data, and depending on user-defined parameters, sort/no sort decisions are made almost instantly. The inFlux nozzle causes the stream to break into droplets in a predictable fashion. The inFlux selectively places an electric charge on individual droplets containing desired particles to be sorted. High voltage plates then deflect the individual droplets from the stream for separate collection.

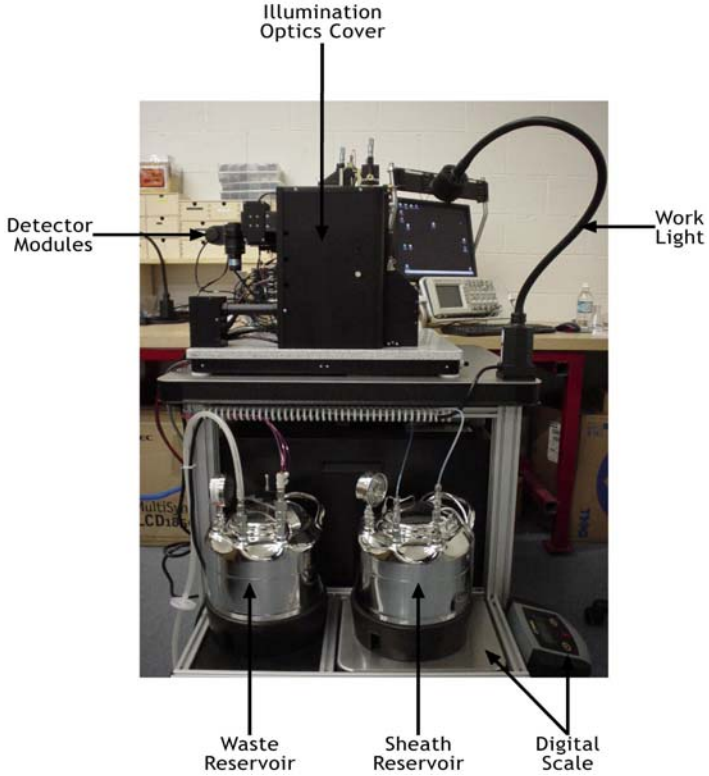
2.2 Pictorial Tour

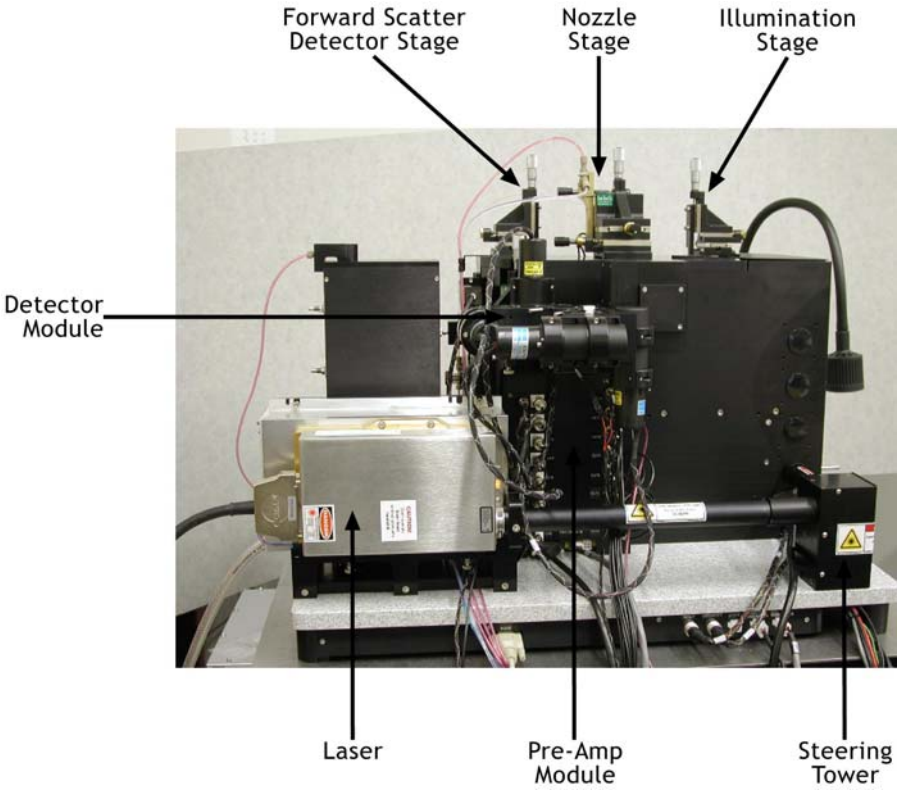
The following photographs provide a brief tour of the inFlux and introduce the operator to subsystem and component terminology.

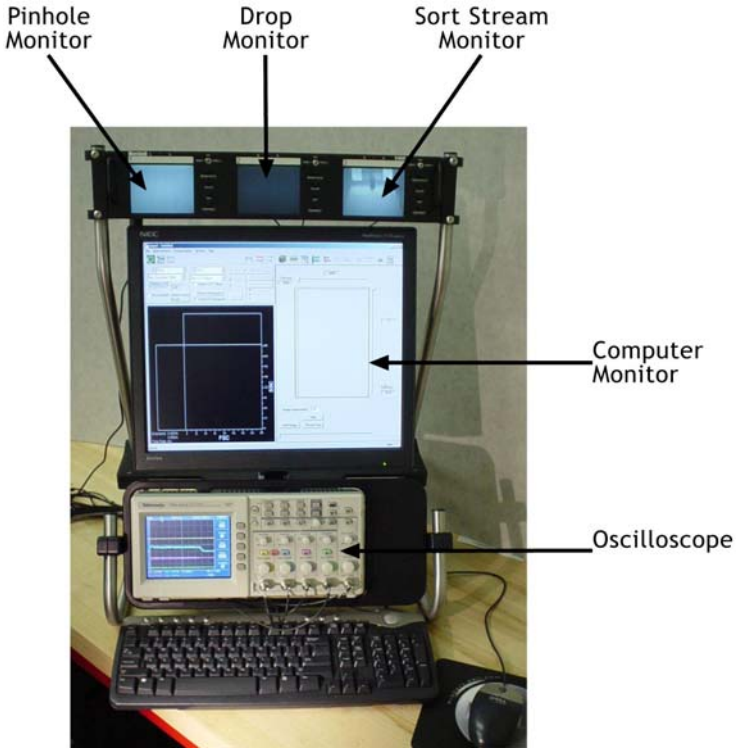





Air Detector
Sample Valve
Air Switch
Sample Stopper
Sample Offset
Boost Button
Sample Station
Fault LEDs
Sample Lever
Cooling (Optional)


Illumination Optics Cover
Detector Modules
Work Light
Waste Reservoir
Sheath Reservoir
Digital Scale


Forward Scatter Detector Stage
Nozzle Stage
Illumination Stage
Detector Module
Laser
Pre-Amp Module
Steering Tower


Pinhole Monitor
Drop Monitor
Sort Stream Monitor
Computer Monitor
Oscilloscope

2.3 Functional Subsystems

This section briefly describes the primary functional subsystems of the inFlux and lists the main components of each subsystem.

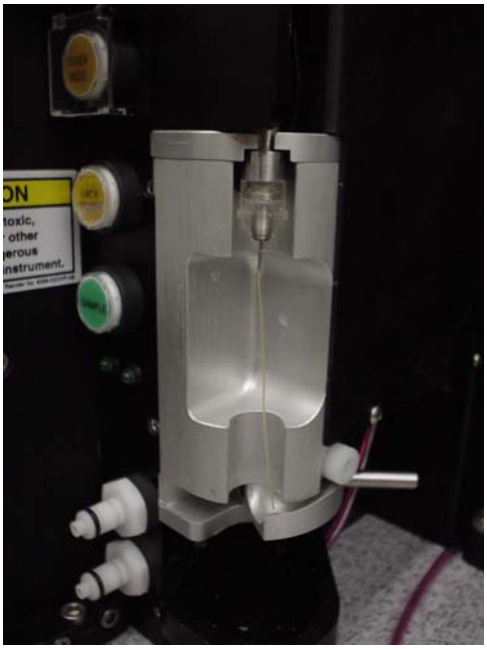
2.3.1 *Fluidics*

The fluidics subsystem includes the components necessary for sample introduction, sheath fluid management, pressure regulation and monitoring, and waste fluid recovery.

Sample introduction involves placing a stream of sample particles, typically suspended in saline, in the center of a larger stream of sheath fluid (saline). Then, under carefully regulated pressures, this stream is forced through a tiny orifice in a nozzle, producing a fine, fast-moving jet of fluid, with sample particles in the center.

The main components of sample introduction are the sample station, the sample line, and the nozzle assembly

The sample station includes a tube holder for loading sample tubes, a sample valve, an air detector and buttons for controlling the sample valve marked SAMPLE, BACKFLUSH, and OVERRIDE.



The sample line delivers the sample from the sample station to the nozzle assembly. The sample line is made from PEEK tubing and has an inner diameter of 254 micrometers. It is designed to minimize sample carryover and is easily replaceable.

Sample is delivered to the nozzle assembly, where it enters the center of a stream of sheath fluid. The nozzle assembly also includes a piezoelectric element used to regulate droplet formation.

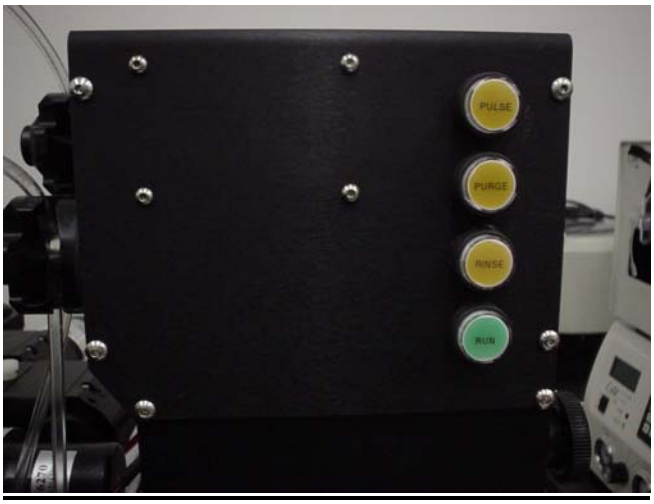
Sheath fluid is so named because the sample fluid is surrounded by a sheath of saline as it is forced through the nozzle, causing the sample to be centered and lined up in the jet emitted from the nozzle tip.

The main components of sheath fluid management are the Sheath Reservoir, a digital scale for monitoring the level of fluid in the Sheath Reservoir, the sheath line and sheath valve, and the nozzle assembly.

Pressure regulation encompasses applying an adjustable level of air pressure to the sample tube and to the Sheath Reservoir. It also involves a means for monitoring the sample and sheath pressures.

The main component of pressure regulation is the Pressure Console and several pressure lines.

Valve mode for the sheath and purge valves is controlled by buttons at the top of the pressure console. Available modes are: *RUN, RINSE, PURGE, PULSE,* and *OFF*. The valve mode buttons toggle the mode on and off. Note the run mode must first be turned off before switching to other modes.



Waste fluid recovery involves retrieving all fluids that have been run through the inFlux, except those that have been separately collected during the sort process.

The main components of waste fluid recovery are the waste drains and buckets, the purge line and valve, the waste lines, the Waste Reservoir and the vacuum pump.

2.3.2 *Illumination*

The illumination subsystem includes all the components necessary to apply a focused beam of laser-emitted light to the stream exiting the nozzle tip.

The main components of the illumination subsystem are the laser(s), protective shields and guards, laser shutters and the shutter interlock system, alignment prisms and/or mirrors, iris diaphragms, and focusing

lenses mounted on linear stages.

2.3.3 *Detection*

The inFlux detection subsystem is modular and open in its design. Its purpose is to spectrally separate and quantify the intensity of light emitted from the particle stream as a result of fluorescence when excited by laser radiation. The detection subsystem also includes the components necessary for light scatter intensity measurement.

The main components of the detection subsystem are image block assembly, and the detector modules. The image block assembly contains the objective lens, the pinhole mirror and prisms for directing the light into the detector modules. The detector modules contain band-pass filters, dichroic mirrors and photomultiplier tubes (PMTs).

2.3.4 *Signal Processing*

Signal processing encompasses all of the electronics components required to control PMTs and amplify and process PMT signals into data that can be monitored by the operator, retrieved and analyzed by Spigot, and used by the sort electronics subsystem.

The main components of signal processing are the logarithmic and linear pre-amplifiers (log and lin preamps) and analog to digital converters (ADCs).

2.3.5 *Sort Electronics*

The sort electronics subsystem contains the electronics components necessary for sorting particles. This process includes transferring sort gating information from Spigot into the hardware look-up tables. These tables use data provided from signal processing to make sort/no sort decisions based on the inputted criteria, and control the electrical components involved in the formation, tracking and sorting of individual droplets.

The main components of sort electronics include circuit boards for hardware look-up tables, counters, piezo drive control, stream deflection, high voltage deflection plates, and control circuitry. Many of these components are housed in the Electronics Console.

2.3.6 *Sample Collection*

After droplets are sorted, the sample collection subsystem is responsible for collecting those droplets. The inFlux enables operators to sort into standard test tubes, a 96-well tray or onto standard microscope slides.

The main component of sample collection is the Well Deposition Unit (WDU), which consists of a two-axis moveable tray powered by stepper motors and controlled by the operator via the Spigot interface. The tray can hold either a 96-well tray or the tray insert, which holds either tubes or slides.

Users may program the WDU to collect in any pattern within its range (for example 384-well tray, 50-ml tube, etc.).

2.3.7 *Monitoring*

The monitoring subsystem enables the operator to observe, in real-time, data acquired by the inFlux, to see feedback on system settings, and to view close-up images necessary for proper adjustment and monitoring of the instrument.

The main components of the monitoring subsystem are the Spigot interface, the oscilloscope and the triple LCD camera monitors: pinhole, drop, and sort stream. Other monitoring components include the pressure readouts, sample station fault LED indicators, the drop position indicator, and the master clock.

[End of Chapter]

Chapter 3 - Safety

Summary: *This chapter alerts operators to known or potential hazards reasonably associated with inFlux operation and outlines safety precautions.*

DANGER	Operation of the instrument poses several potential hazards, including hazardous voltage and current, laser radiation and moving parts. PLEASE read and understand this safety information and this entire manual prior to operating the instrument and follow all recommended safety precautions.

3.1 Hazards

The following table lists the primary potential hazards that may be encountered during inFlux operation. The instrument is labeled with ANSI/ISO-harmonized safety labels, alerting the user to these potential hazards. The alerting symbols contained in these labels are shown in the table.

Type	Potential Hazard	Alerting Symbol
Electrical	Electric shock from high voltage deflection plates, high voltage circuitry and other electrical circuits.	
Light	Laser radiation	
	Ultraviolet (UV) light emission	
Mechanical	Pinching or crushing due to moving parts.	

Pneumatic	Contents of reservoirs and fluidics lines under pressure.	
Heat	Heated air emitted from instrument.	No label

3.2 Safe Operation

3.2.1 *General Electrical*

- As with all electrical equipment, protect against shock by connecting the instrument to an approved grounded power source.
- Do not, under any circumstances, remove the grounding plug from the power plug. Do not use extension cords.
- Do not perform any servicing except as specifically stated in this manual.
- Do not remove any wiring cover or panel with a warning label affixed to it. These covers and panels provide protection against potential electrical shock from components inside.

3.2.2 *High Voltage Plates*

This instrument requires the use of high voltage plates to deflect desired droplets from the stream for collection. Thus, whenever the plates are in use, the potential for electric shock exists.

DANGER	High Voltage. Do not reach into the workspace while the deflection plates are energized! Switch off high voltage plates prior to opening door and reaching into workspace.

3.2.3 *Light Hazards*

Laser Radiation:

This instrument utilizes both Class 2 and Class 3b laser radiation during operation. Follow all standard laser safety precautions.

WARNING	Laser radiation present. Wear suitable eye protection. AVOID DIRECT EXPOSURE TO BEAM.

- For each laser installed with the instrument, follow the laser manufacturer's operating manual and facility-specific laser safety precautions.
- For the safety of operators and other persons near the inFlux system during operation, the instrument is equipped with an

automatic laser shutter interlock system. However, suitable eye protection must still be worn. Do not intentionally defeat the automatic interlock system.

- Do not remove protective covers, guards, or doors with interlocks defeated, unless specifically necessary for adjustment of the instrument AND PROPER EYE PROTECTION IS USED.
- Use of controls, adjustments, or performance of procedures other than those specified in this user's guide may result in hazardous laser radiation exposure.

Ultraviolet Light

The instrument has UV light in the workspace for sterilization purposes. During normal operation, the bulb is covered to prevent exposure.

CAUTION	Do not look directly at the UV bulb when it is illuminated. Do not remove the UV bulb's protective cover.

3.2.4 *Mechanical*

Operators potentially can be exposed to mechanical pinch or crush hazards due to moving parts, primarily from the WDU and the instrument's doors.

- Do not remove any guards or covers with labels alerting operators to a mechanical hazard.
- Use caution when operating doors to avoid injury from door linkages.

Well Deposition Unit:

CAUTION	The WDU contains moving parts that present a mechanical crush or pinch hazard during operation. Do not reach into workspace while the unit is operating. Do not remove any of the WDU's guard covers; this will expose motors and sharp-edged moving parts.

3.2.5 *Pneumatic*

Pneumatic hazards present during instrument operation are pressurized air and vacuum.

- Ensure that facility pressure and, if applicable, vacuum sources are appropriate and are safely connected to the instrument.
- Be familiar with the facility and know how to quickly shut off air and vacuum in the event of a problem.
- Use caution when near vacuum sources.

CAUTION	The Sheath Reservoir, sheath lines and sample lines are pressurized during operation. **Do not operate the instrument at more than 100 psi.** Use caution when handling Sheath Reservoir connections and when relieving reservoir pressure with the pressure relief valve.

3.2.6 *Biohazard*

CAUTION	Do not introduce any toxic, biohazardous or other potentially dangerous substance into the instrument.

Responsibility for disregarding this caution rests solely with owners and operators of the instrument. As with all research-grade equipment:

- Observe universal biohazard safety precautions and utilize standard laboratory procedures.
- Operate instrument only with doors fully closed to guard against aerosols, splashes and spills.
- Keep instrument workspace and table clean. Do not store liquids on the instrument or table.

[End of Chapter]

Chapter 4 - Operating Procedures

Summary: *This chapter contains detailed step-by-step instructions for operators new to the instrument. Information on the use of the Spigot interface application is presented as part of these instructions; however, users may also refer to the Spigot Interface chapter for further details on Spigot.*

4.1 Preparation

4.1.1 *Collect supplies*

Gather all supplies necessary for instrument set-up. These may include: sheath and waste reservoirs, nozzle tip, nozzle back flush reservoir, sample tubes, cotton swabs, paper towels, and microscope slides. Make sure that all supplies are clean.

4.1.2 *Prepare fluidics reservoirs*

Fill the sheath reservoir with up to 7 liters of 0.2 micron filtered clean sheath fluid. Ensure that the waste reservoir is clean and empty. Attach lids and quick connect fittings from instrument fluidic lines to the reservoirs. Note that each reservoir port is unique, so that each fitting connects to only one port.

4.2 Power-up

4.2.1 *Power up Fluidics*

Start fluids early. Fluids take from 30 minutes to one hour to fully stabilize. It is therefore important to start the fluidics as early as possible.

Set Valve Mode to Off. Make sure that the valve mode is set to off (no mode buttons on pressure console are illuminated – See section 2.3.1).

Choose Pressure and Frequency settings. The inFlux is capable of running with several different nozzle tip sizes, most of which have many different pressure/frequency combinations. The inFlux droplet rate (frequency) is set by the master clock and ultimately determines the practical upper limit for running particles when sorting. A good rule of thumb is to have a droplet rate that is about ten times the sample rate, if possible, in order to minimize coincident (two particles in one droplet) events.

See Appendix B for a chart of available pressure/ frequency settings.

Pressurize Sheath Reservoir. Close the pressure relief valve on the sheath reservoir. Turn on air supply by flipping up the **AIR** switch on the Pressure Console. Observe the gauge on the sheath reservoir to ensure that it is being pressurized (below left). Reseat the lid of the sheath reservoir if the pressure in the reservoir does not increase.

Sheath and Waste Reservoir gauges

Set the Sheath and Sample Pressures. The pressure console includes four regulators – Sheath, Sample, Sample Offset, and Boost. Both the sheath and sample regulators can regulate an input pressure (of at least 20 PSI over the desired setting) of 3-100 PSI, although operating pressure should be kept below 80 PSI for safety. The sample offset regulator adds 0-5 PSI to the sample regulator, allowing fine control of the sample rate. The boost regulator is set to add 5 PSI to the sample pressure when the boost button is depressed and should not usually be adjusted.

Use the regulator knob labeled *SHEATH* on the right side of the pressure console to set the sheath pressure to the desired value.

To balance the sample pressure with the sheath, follow these steps:

1) Dial out (decrease) the regulator knob labeled sample offset on the front of the pressure console until it no longer lowers the sample pressure readout on the pressure console.

2) Use the regulator knob labeled *sample* on the side of the pressure console to adjust the sample pressure until the *sample pressure readout is 1 PSI lower than the sheath pressure readout*.

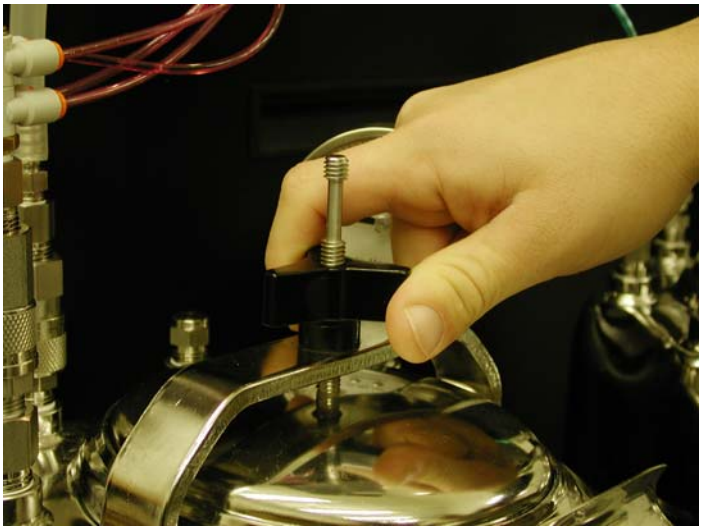
3) Use the *sample offset* regulator knob to *increase the sample pressure readout to 1 PSI greater than the sheath pressure*.

This process ensures that the sample offset regulator is adding about 2 PSI to the total sample pressure, and also leaves the sample to sheath differential pressure at about 1 PSI – typically a good starting point when running particles.

Sample input and fluidics valves

Apply vacuum. Turn on the vacuum pump or other vacuum supply. Read the gauge of the waste reservoir and ensure that it reads at least 5” Hg (above right). If less than 5” Hg is observed after the tank has a few moments to come to pressure, tighten lid. It is necessary for the waste

reservoir lid to be tightened as much as possible without using a wrench.



Tighten Waste Reservoir lid

4.2.2 *Start Lasers*

Most lasers require at least 30 minutes of warm-up time. Close all laser shutters on the instrument and start all lasers in accordance with the manufacturers' procedures. Typical laser control units are shown below. Control units have key switches to prevent unauthorized or unintended use. Power output of the laser may be set to the desired level, or the laser may be put into standby mode, if desired.

Laser controllers and computer

4.2.3 *Start Computer*

Turn on the computer.

4.2.4 *Power-up Electronics Console*

Make sure that all switches on the Control Panel are in the OFF (down) position.

Control Panel

Turn on the Electronics Console by flipping up the main power switch (see right) in the lower left corner of the console. The switch will be illuminated when power is on. All peripheral monitoring devices will power up when the Electronics Console is turned on.

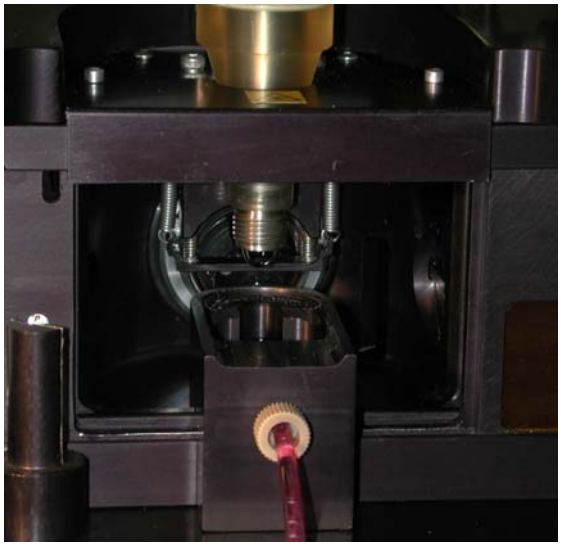
4.2.5 *Start Spigot*

Launch Spigot either from a desktop shortcut or from its directory: C:/program files/spigot X.X.X/spigot.exe.

4.3 Stream Generation

4.3.1 *Flush system*

Place the flush bucket under the nozzle. Recheck that the waste reservoir has an adequate level of vacuum applied to it.



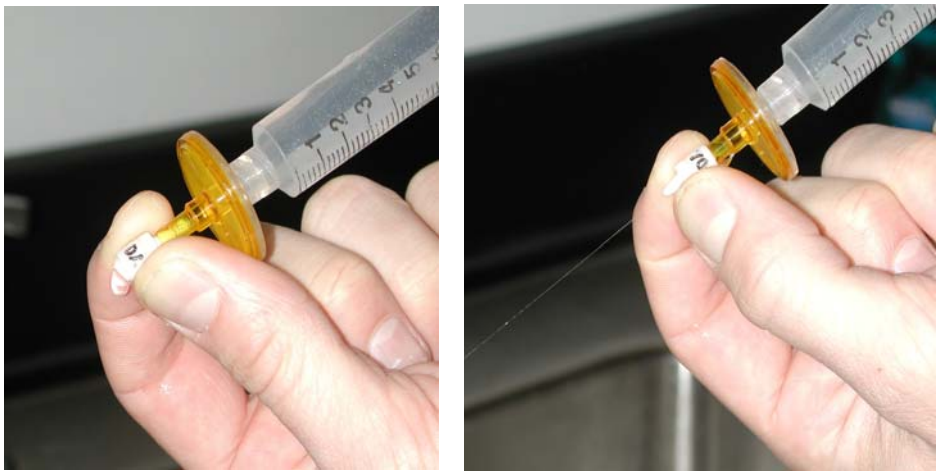
Flush bucket in place with nozzle nut removed

Press the RINSE button to open the sheath and purge lines. Take care

not to overfill the flush bucket, if necessary reduce sheath pressure to 15-20 PSI to prevent overfilling.

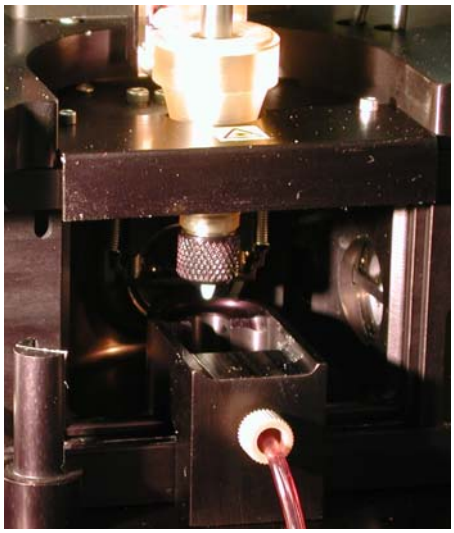
Run fluid through the lines for 1 - 2 minutes. Ensure the lines are mostly full of fluid. Press the RINSE button to close the sheath and purge valves and stop the flow. Clean and attach nozzle tip

Initially clean the nozzle tip by sonicating it for approximately 5 minutes. Then use a syringe to further clean by flushing 0.2-µm filtered water (de-ionized, distilled, or other), sheath fluid, or mild detergent through the nozzle tip in two steps as shown below, flushing “backward” through the tip first. Take care not to let any contaminants enter the nozzle tip.



Clean nozzle tip with syringe from both directions

Install the nozzle tip by carefully placing the nozzle tip in the nozzle nut (large tweezers can be used to avoid contamination). Be sure that there is an o-ring around the nozzle tip inside the nozzle nut. Screw the nozzle nut onto the nozzle and tighten as much as possible *without using a wrench*.



Nozzle tip and nut installed

4.3.2 *Start a stream*

Press RUN to open the sheath valve and start a stream. Be sure to return the sheath pressure to the desired operating pressure if adjusted during rinsing.

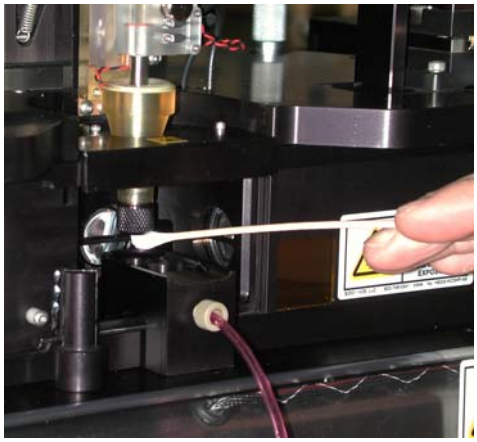
4.3.3 *Back flush and de-bubble*

Make sure that a sample tube is not loaded and that the sample tube lever is in the open position. Back flush the sample line by pressing BACKFLUSH on the sample station. Allow the sample line to drip sheath fluid for about 30 seconds. Press BACKFLUSH again to close the sample valve and complete back flushing.

Press RUN to deactivate the run mode which will close all valves. Purge the nozzle of bubbles by placing the clean nozzle purge reservoir filled with 0.2-μm filtered sheath fluid atop the flush bucket.

Nozzle purge reservoir rests in place on flush bucket

Ensure that the nozzle tip is submerged into filtered sheath fluid in the nozzle purge reservoir. Press the PURGE button to open the purge valve and pull fluid up through the nozzle tip and purge the system of air. Bubbles should be observed in the purge tubing as they are pulled up through the nozzle. When all bubbles have traveled past the purge valve press the PULSE button to free any other bubbles lodged in the nozzle. Allow freed bubbles to travel past the purge valve and PULSE again. Continue pulsing and purging until no sizable bubbles are freed from the nozzle. If necessary, add sheath fluid to the nozzle back flush reservoir (a syringe with filter works well) to keep the nozzle tip submerged throughout this process.



Swab excess fluid from sides of nozzle tip

Remove the nozzle back flush reservoir and use a cotton swab or similar to remove excess fluid from the outside of the nozzle tip.

Ensure that the stream is emitted straight out of the nozzle tip. If the stream is even slightly askew, return to the top of this subsection and repeat cleaning the nozzle and flushing the system.

4.3.4 *Align stream*

Make sure that an Erlenmeyer flask is placed under the stream drain. For most instruments this is accomplished by putting the Erlenmeyer flask in the WDU and setting the WDU position to *Safe*.

*WDU tray in **PRESENT** position*

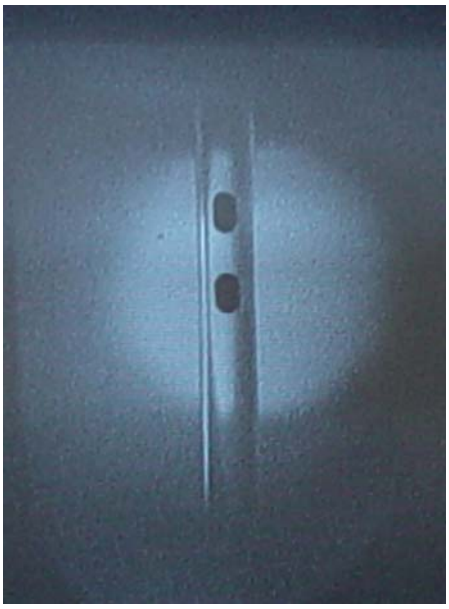
Remove the flush bucket so that the stream passes through the illumination chamber into the sort chamber. Flip the **ILLUM** switch on the Control Panel to **ON** to illuminate the stream near the drain. The stream may or may not land in the stream drain, but should be captured by the Erlenmeyer flask if it misses the stream drain. If not, take measures necessary to prevent spills.

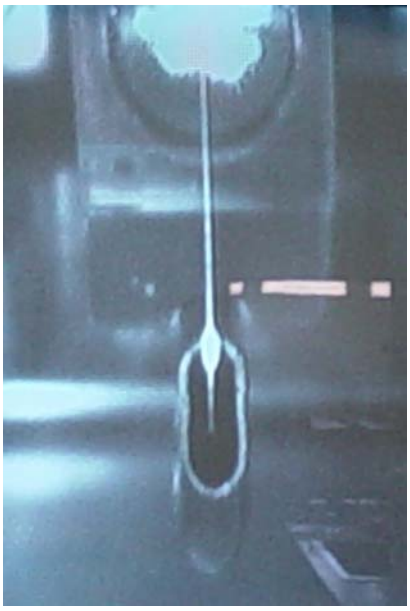
Alignment of the stream consists of: (1) bringing the stream into the focal point of the microscope objective and (2) ensuring that the stream is properly positioned in the drain. Use the pinhole monitor and the sort stream monitor for primary visual feedback while making adjustments to the nozzle stage.

(1) Bring stream into focus. Use the x-axis (focus), y-axis (left-right) and z-axis (up-down) micrometers of the nozzle stage to focus and position the stream in the pinhole monitor as shown below left. The stream should be centered on the pinholes, sharply focused, and the nozzle tip should be barely visible in the top of the monitor. It may be helpful to use the halogen lamp to illuminate the stream in the chamber for this procedure.

(2) Position stream. Once the stream is properly focused in the pinhole monitor, use the Θ-angle and Φ-angle micrometers of the nozzle stage to aim the stream at the drain in the sort stream monitor.

Iterate several times between steps (1) and (2) until the stream is focused and positioned in the pinhole monitor and also aimed at the drain in the sort stream monitor.





Right: Stream focused in pinhole camera image

Left: Stream aimed at drain

4.4 Droplet Formation

Droplet formation is achieved by using a piezoelectric element to focus an acoustic wave into the stream at the nozzle tip. The stream may need to run for about 10 minutes before droplet formation will become stable.

4.4.1 *Turn on the Piezo drive*

Flip up the **PIEZO** switch on the Control Panel. Rotate the **PIEZO** knob to

an appropriate amplitude setting (typically, this setting is approximately 3 for low pressure settings and 6 for high pressure / large tip settings. If the piezo amplitude is set too high, the waveform of the jet will interfere with the forward scatter signal. If the amplitude is too low, the Break-Off Point (BOP) will be instable, and sort streams may spray when sorting large particles. Fine-tuning of the piezo amplitude will be completed later when optimizing the BOP.

4.4.2 *Choose a Droplet Frequency*

Adjust the frequency on the master clock according to the pressure and tip size being used (see Appendix B for approximate optimal settings). Note that the droplet frequency is 1/16th of the master clock frequency.

4.4.3 *View the Break-Off Point (BOP)*

Adjust the up-down translation stage of the drop camera so that the BOP can be seen in the drop monitor.

Drop camera image

4.4.4 *Optimize Droplet Rate*

Use the master clock to scan frequencies near the suggested optimum frequency for the pressure and tip size being used. Choose a frequency that causes the break off point to be as high (short) as possible. It may be necessary to adjust the camera height and piezo amplitude during this procedure so as to keep the BOP visible in the drop monitor.

4.4.5 *Clean, Close and turn on PLATES*

Important: Clean the deflection plates by wiping them with a kimwipe or other towel wetted with DI water. Wipe the plates again with a dry wipe to ensure that there is no fluid on the plates before closing them.

Swing the deflection plates into their closed positioned and tighten their thumbscrews to hold them in place.

Deflection plate and thumbscrew

Close the sort cavity door and switch on the high voltage plates by flipping the **PLATES** switch on the control panel up. Note that a red LED on the control panel will illuminate to indicate that the plates are on.

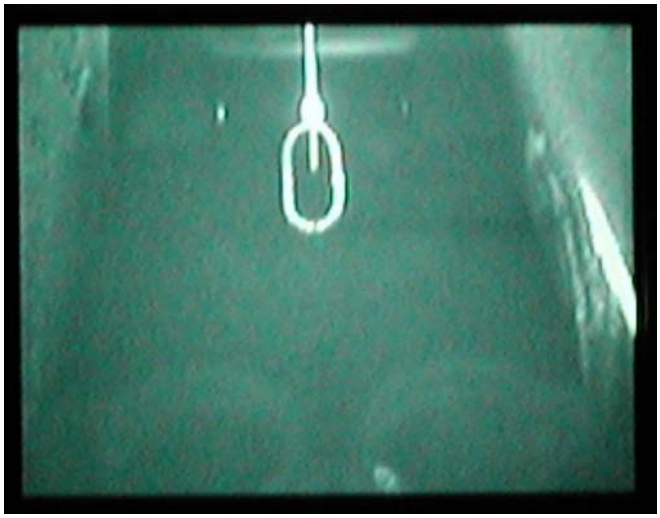
4.4.6 *Turn on STREAM DEFLECTION*

Flip up the **STREAM DEFLECTION** switch on the control panel. Set the **STREAM DEFLECTION AMPLITUDE** knob to about ¼ of its full range for now.

4.4.7 *Optimize Break Off Point*

Create test sort streams and tune the piezo amplitude optimize the BOP

Create left and right test sort streams by flipping up the **TEST LEFT** and **TEST RIGHT** switches on the control panel. Sort streams should now be visible in the sort stream monitor. Use the deflection amplitude knob to adjust the sort streams so that they clear the drain as shown below.



Sort stream image, with side streams

Phase the droplet formation with the drop charge using the following important method:

Flip up the **1-1 ½** switch on the control front panel. Adjust the **PIEZO AMPLITUDE** knob until the sort streams are maximally deflected, with the **1-1 ½** switch in the up position. Ignore small satellite streams near the main waste stream when the **1-1 ½** switch is up.

Use the drop monitor to note that the piezo amplitude can be increased or decreased until the next or previous drop is deflected. Once the desired drop is deflected optimally carefully note the appearance of the droplet formation at the BOP. View and note the connectedness of the "ligament" or small satellite droplet after the last connected drop. It is crucial that the droplet waveform remains constant while sorting. It is sometimes necessary to adjust the **PIEZO AMPLITUDE** knob while sorting to keep the waveform of the jet at the BOP (connectedness of ligament / satellite drop) constant.

Flip off **1-1 ½.**

4.4.8 *Set STREAM FOCUS*

After flipping the **1-1 ½** switch down, view the sort stream monitor and adjust the **STREAM FOCUS** knob on the control panel until the main center stream (stream going into drain) is as tight as possible (minimize satellite streams). Turn the **TEST LEFT** and **TEST RIGHT** switches 'off'.

4.4.9 *Set STREAM DEFLECTION amplitude*

The proper setting of deflection amplitude results in a sort stream that falls in the center of a sort tube during 2-way sorting.

Place a tube in the left receptacle of the WDU insert. Place a microscope slide over the tube. From Spigot's **SORT TRAY** screen send the WDU to

the *2-Tube*⇨*Sort Ready* position.

Quickly flip up and down the **TEST LEFT** switch on the control panel to put a small droplet of water on the slide.

Send the WDU to *Present*. Check the slide to see if the droplet would have landed in the center of a collection tube. If not, adjust the **STREAM DEFLECTION** amplitude, and/or use Spigot to offset the sort ready position and repeat this test until the stream is deflected to land in the bottom of a sample collection tube.

Alternatively, or in conjunction to altering the **STREAM DEFLECTION** amplitude, the sort ready position for each sort procedure may also be offset and saved in Spigot on the **SORT TRAY** screen.

If 96-well sorting is desired, follow a similar procedure except use a 96-well tray insert and the *96-Well Sort* mode for the WDU mode.

4.5 Illumination

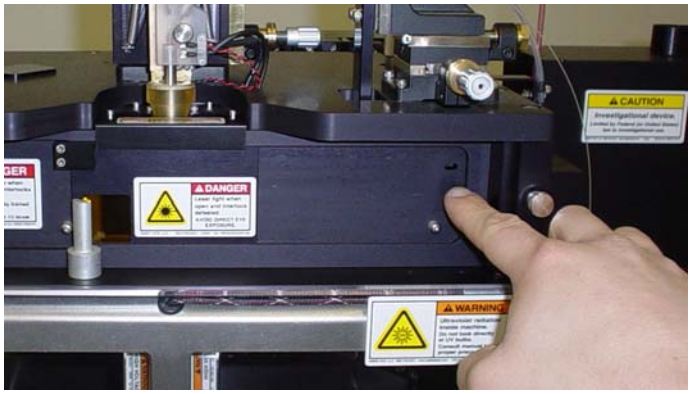
Illumination consists of steering the laser paths so that they intersect the stream at the proper locations. The following steps should be repeated for each illumination laser.

4.5.1 *Open laser shutter*

Close the chamber door slide and put the chamber lid in place. Open the manual laser shutter on the left side of the sort head.

Manual laser shutter, in open position

The instrument is also equipped with an automatic laser shutter interlock system. When the system is armed, opening the chamber door slide will cause the automatic shutter to close. It will not reopen unless two conditions are met: 1) the chamber door slide is closed, and 2) the system is reset by placing a finger in front of the reset detector. The reset detector is behind a small hole in the upper right corner of the chamber door, as shown below.



Laser interlock system reset aperture

WARNING	The laser interlock system does NOT eliminate the need for operators to wear suitable eye protection. It is possible that a bright light pointed at the chamber area will cause the laser shutter to be opened even though the chamber door slide is open..

4.5.2 *Steer laser to stream*

Note: Most lasers will show a glow in the pinhole monitor when they strike the stream. All lasers will scatter off of the stream and create a horizontal laser scatter line across the chamber and the chamber door window. This procedure can be more challenging with UV lasers due to their invisibility. Fine-tuning of the laser will be completed later, when running calibration particles.

Use the illumination stage's y-axis (sweep) and z-axis to aim laser at both the center of the stream and at the height of the appropriate pinhole. Adjust the y-axis micrometer of the nozzle stage so that the intensity of the illuminated band on the stream is maximized. Adjust the z-axis micrometer so that the appropriate pinhole is illuminated. Again, this is an iterative process. The laser will not need to be focused except for special applications.

Repeat this process for all laser paths.

4.6 Sample Introduction

4.6.1 *Load sample tube*

Use only Falcon 35-2063 5 mL sample tubes. Cytopeia recommends that all samples be filtered to 40 µm, or about half of the nozzle tip size, to prevent nozzle clogs and ensure optimal sorting.

Make sure that the Fault LEDs are both lit. If the Left LED is not lit, then there is fluid in the air supply line – close the Sample Lever without a tube loaded to blow any fluid out. If the right LED is not lit, then there is air in the sample line – open the sample lever and press BACKFLUSH to purge the sample line of air.

Fill sample tube with up to 3 mL of sample. Load sample tube into sample tube holder and lock tube in place over the stopper using the lever. Make sure that the sample lever is completely closed. Take care not to damage the sample tube.

Locking sample tube in place

CAUTION	Use care when removing the sample tube; the sample tube is usually pressurized and may spill or splatter sample fluid. To properly remove the sample tube, move the lever slowly backward until the tube is pushed down into the recessed area of the lever. Pressure is then released and the tube may be removed safely.

4.6.2 *Open SAMPLE VALVE*

Check that the sample pressure is set at about 1 PSI over the sheath pressure. Check that both small green LEDs are lit on the front of the fluidics station, if not backflush the sample line and/or close the sample lever without a sample tube loaded until both are lit. Press SAMPLE to open sample valve and begin running sample. Boost may be depressed for a few seconds quicken sample delivery to the nozzle tip.

4.6.3 *Run sample at a low rate*

Aim the laser so that it is just above the center pinhole, in-between

pinholes for multi-laser systems. When running bright calibration beads or cells at a low flow rate a narrow sample core can be viewed in the pinhole monitor. If the flow rate is too high, the sample core will be very large, approaching the size of the jet. If no beads are observed flashing in the stream then the flow rate is too low.

Pinhole camera image, with beads showing in stream

The flow rate of the sample is determined by the sample pressure setting. At a sample pressure of 1 PSI over sheath pressure a low flow rate is usually achieved, however this setting depends on the concentration of the sample.

When first running a sample you may wish to boost the sample pressure temporarily in order to get sample to the jet quickly. Hold down the boost button to boost the sample to 3-5 PSI over the sheath.

Adjust the sample pressure until a low flow rate is achieved.

Adjust the y-axis of the illumination stage so that the brightest signal is observed in the pinhole monitor. Adjust the x-axis of the nozzle stage so that the signal is tightest (spatially) in the pinhole monitor. Iterate between these steps until the sample core is as narrow and bright as possible.

Use the z and y-axes of the nozzle stage to place the sample core in the center of the pinhole.

The sample valve may be closed by pressing SAMPLE if desired at this point to preserve the sample until data acquisition parameters are set up.

4.7 Data Acquisition

Spigot software is used for most all data acquisition controls. Spigot allows control of PMTs and allows custom gating schemes to be developed and saved in configuration files. It is recommended that a configuration file be saved for each protocol run on the inFlux. See the

chapter on Spigot Interface for detailed descriptions of Spigot functions. This chapter will refer to fluorescence channels as FL1-FL7 and forward and side scatter as FSC and SSC.

4.7.1 *Open a Spigot Configuration File (*.sco)*

If a Spigot configuration file has already been saved for alignment particles, open that file and skip the steps outlined below for configuring Spigot. Otherwise, follow the directions below to set up a new configuration and save the settings in a new *.sco file by selecting **FILE: SAVE CONFIGURATION FILE** in Spigot.

4.7.2 *Activate PMTs*

Activate all PMTs that will be used during alignment. It is crucial that the FSC PMT is activated since it is the signal that is (typically) used for the system trigger. Thus, no data will be acquired unless the FSC PMT is activated.

4.7.3 *Choose parameters to plot*

Choose parameters to plot in the Spigot's left display as described in the Spigot Interface chapter. Typically, FL1 is plotted vs. FSC and these instructions will detail how to set-up and align to those two parameters. Use a similar method for plotting other or more parameters.

4.7.4 *Set initial PMT gains*

The inFlux system is shipped pre-configured for linear (lin) signal amplification for scatter channels and logarithmic (log) amplification for fluorescent channels. These settings can be changed on the Patch Panel on the front of the electronics console. Spigot can also be set to either log or lin for any channel as a visual reminder of the controlling hardware setting and to display logarithmic scales on data plots.

As PMT gains are increased the system will begin to acquire pulse data. The oscilloscope is used to monitor the pulses and is very useful for setting initial PMT gains. From Spigot's **SIMPLE CONFIG** screen set the *Scope Display* ⇨ *CH1* and *CH2* displays to FSC and FL1 (usually channels 1 and 3).

Use Spigot to increase the FSC gain until pulses of about 5 V can be detected on the oscilloscope. Do the same for FL1.

Since the range for pulse height measurements in Spigot is 0-10 V pulse heights of about 5 V should correspond to data that is acquired in about the middle of a FL1 vs. FSC plot in the left display.

Make sure that data are being acquired by Spigot and adjust that PMT gains so that the data are not off-scale.

4.7.5 *Set Trigger Level and Event Trigger Delay*

Trigger Level sets the threshold for the lowest detectable signal and it set

off of the system trigger (usually FSC). Its intended use is to eliminate noise. It is usually best to keep the *Trigger Level* as low as possible since the system will not detect any events that fall below the threshold, and those events may end up in the sorted sample.

With multi-laser systems it is necessary to set the *Event Delay* for each additional laser. Align additional lasers using the pinhole monitor and the oscilloscope. Adjust the *Event Delay* from the menu until the signals from additional lasers fall into appropriate "bins" as displayed on the third oscilloscope trace (labeled as the ALL cable). Finally make sure that additional laser channels are set to the appropriate events (1, 2 or 3) in the SIMPLE CONFIG screen. Typically, *Event Delay* will only need to be changed if the sheath pressure of the system is changed.

4.8 Alignment

Fine-tuning of the inFlux involves positioning the sample core at the focal point of the objective lens in the pinhole, illuminating the sample core optimally, and aligning the FSC. PMT gains may need to be adjusted as the system is tuned so as to keep the data on scale.

4.8.1 *Focus light emission from sample in stream*

Open sample valve and ensure that sample is flowing at a low rate. Ensure that the sample core is focused, maximally illuminated, and in the center of the pinhole using the pinhole monitor, nozzle stage and illumination stage as described earlier in this chapter.

4.8.2 *Optimize a fluorescence channel*

While a FSC signal must be detected first (if it is the system trigger), it is important to first align to a FL or SSC channel since the objective lens is fixed while the forward detector stage allows adjustment.

Some flow cytometer operators prefer to align the FL signals using linear amplification. To do so, move the BNC connector on the front of the electronics console from LOG to LIN for the appropriate channel. Remember to set the channel back to LOG after tuning and use Spigot to re-adjust the PMT gain appropriately.

Adjust the y-axis of the illumination stage and use the oscilloscope pulse monitor and the left spigot screen to maximize the signal from the FL1 detector. The pulse should be as high as possible and the signal in Spigot should be maximized. Remember to ignore the FSC signal while tuning the FL channel.

4.8.3 *Optimize forward scatter signal*

Once the FL signal is optimized, the FSC signal can be optimized. Use the y- and z-axes of the forward scatter stage to maximize the signal from the forward scatter detector.

4.8.4 *Optimize additional laser paths*

After the first laser path is aligned for fluorescence and FSC, additional laser paths can be aligned for fluorescence using their respective illumination stages as described above.

4.9 Sort Gating

Sort gates can be defined via the Spigot interface. Up to 12 (six left and six right) 2-parameter sort windows can be defined at a time. Sort windows are two-dimensional sort gates that allow the operator to select portions of data to be sorted. Many options, such as sort filtering, are available. These instructions will describe how to set up a basic sort window as well as a few advanced features. The Spigot Interface chapter also contains reference information.

4.9.1 *Draw Sort Windows in Spigot*

To draw a sort window (SW) in Spigot first click on **SORT**. The right display will contain six squares corresponding to the instrument's six Look-Up Tables ("LUTs"). Highlight LUT 1 by clicking inside the square of LUT 1. The border around LUT 1 will turn orange, indicating that LUT 1 is selected for editing. Choose the parameters for the SW (i.e. FL1 vs. FSC) by changing the *X* and *Y* axes selection on the left display. The LUT will inherit the axes of the left display's plot when axes are changed.

In the left toolbar's sort window function area, choose whether the gate will be a left SW, a right SW, or both, by clicking *Left*, *Right* or *Both*. Next click *Draw Window* and draw a SW on the left display. A SW can be drawn as a rectangle, ellipse or polygon. To draw a rectangular or elliptical SW click on *Rectangle* or *Ellipse* and left click and drag on the left display. To draw a SW of any shape (polygon), single left click on the left display, move the mouse to another position and single left click again and continue the process until the desired shape is drawn. Right click to close the polygon and finish drawing the SW. Once the SW is drawn on the left display it will also appear in the LUT on the Right Display if *Show Sort Window* is checked in the right toolbar. Data that have a left SW drawn around it will become green, and data that have a right SW drawn around it will become red. Data that are selected for both right and left sorting will become yellow and will not be sorted.

Additional LUTs can now be selected, and different parameters may be selected for SW drawing. For example, LUT 2 may be chosen to display FL2 vs. SSC. By drawing another SW around these new parameters it is possible to detect a particle with four desirable characteristics (e.g., FSC, FL1, FL2, FL3; it may fluoresce green, have particular forward and perpendicular scatter values, and not fluoresce red).

In this fashion up to 12 parameters can be set for both right and left sorting (depending on the number of photomultiplier tubes included in the

particular inFlux configuration). SWs follow “and” logic; as additional SWs are created in multiple LUTs, only data that is selected in *all* SWs will be sorted. Data that are selected for left or right sorting in *all* SWs will appear green (left sort) or red (right sort) in the left display.

An operator may also choose data outside of a SW to be sorted by again selecting the LUT in the Right Display, drawing a SW in the left display, clicking on *Left* or Right and clicking *Outside* in the left toolbar. This allows for “not” logic to be used when multiple SWs are drawn in multiple LUTs.

Overall sort statistics are displayed on the left display, while individual statistics for each LUT are displayed next to each LUT. LUT statistics report the percentage of particles chosen for left sort, right sort, neither or both for that particular LUT. Overall sort statistics report the combined percentage of particles selected for left, right, neither or both sort when SWs have been drawn in multiple LUTs.

The *Reset* button (in the sort window function area) can be used to reset SWs drawn in a particular LUT window by selecting the LUT and clicking on *Reset*. All LUT SWs can be reset by clicking the *Reset All* button on the right toolbar). Individual LUT SWs can also be made active or inactive by selecting the LUT and clicking the *Active* button in the sort window function area. SWs may be moved or stretched by clicking on the *Modify* button.

4.9.2 *LUT/Classifier Filters*

Spigot allows data filtering based on SWs for easy identification of populations and subpopulations across many parameters. Two types of filtering are possible: LUT filtering and Classifier filtering. When LUT filters are activated, the left display will only show data that are inside SWs drawn in the LUTs. LUT SWs can be filtered through in any combination, alone, or not at all. When a classifier filter is selected the left display will only display data that have been selected for sorting in *all* LUT displays (i.e. the actual data to be sorted).

To apply a LUT filter click on *Show LUT Filters*. A small rectangle of six boxes will appear in the left display. These six squares represent the 6 LUTs and by clicking on one of the squares the data will be filtered through any SW drawn in that particular LUT. Multiple LUTs can be filtered through in combination by clicking on more of the squares.

Classifier filters can be applied by changing the left toolbar’s drop down menu selection from *No Classifier Filter* to the desired classifier filter setting.

4.10 Analyzing

Once the system is tuned, data may be analyzed. Spigot provides real-time analytical tools described in the Spigot Interface chapter. For

detailed statistical analysis Spigot allows data to be stored in the *.fcs file format that can be read and manipulated with a separate analysis program such as Flowjo.

4.10.1 *Save *.fcs files*

To save data to an *.fcs file from Spigot, refer to **LIST TOOL** and **QUICK LIST** sections in the Spigot Interface chapter.

4.11 Compensation

The inFlux is capable of unlimited real-time hardware compensation for up to 16 parameters. Once compensation values have been applied the user may display, gate on, and store both compensated and uncompensated data. Compensation is intended to be used strictly with log amplified data. There are many different methodologies used for compensation, this section will describe two methods for compensating with the inFlux. Both methods begin with the same step:

4.11.1 *Set initial PMT gains*

Run negative (unstained) control particles. These may be unstained cells or blank beads if you are using beads to set up compensation. Decide which (fluorescent) parameters are going to be compensated (based on your experiment / sort), these will be the parameters for which the PMT gain will need to be accurately set.

Alternatively, you may run single color samples mixed with negatives. This method can be advantageous when setting PMT gains for fluorescence channels excited by lower power lasers since negative populations can be harder to identify.

Plot the first fluorescent parameter and adjust the PMT gain so that the mean of the negative population is visible on scale, but most all of the data falls below the first decade. For lower power lasers, such as the 635 nm laser, it may be difficult to find a mean for the negative population. In this case set the gain so that most of the data falls in the first decade. Use the oscilloscope to monitor the data pulses when setting gains to ensure that gains are not set so high that PMTs are saturated (baseline will come up when PMT is saturated).

Continue to plot fluorescent parameters that will be used and set the PMT gains for each parameter as described above.

If using *Method 2 (offline)* and running positive single colors with negative cells, save an *.fcs file for each positive sample that will be used to compensate.

Once PMT gains have been set it is very important the they *not be altered while or after compensating*, as compensation values are directly dependant on PMT gain. Save an *.sco file so that PMT gains can be recalled if inadvertently altered.

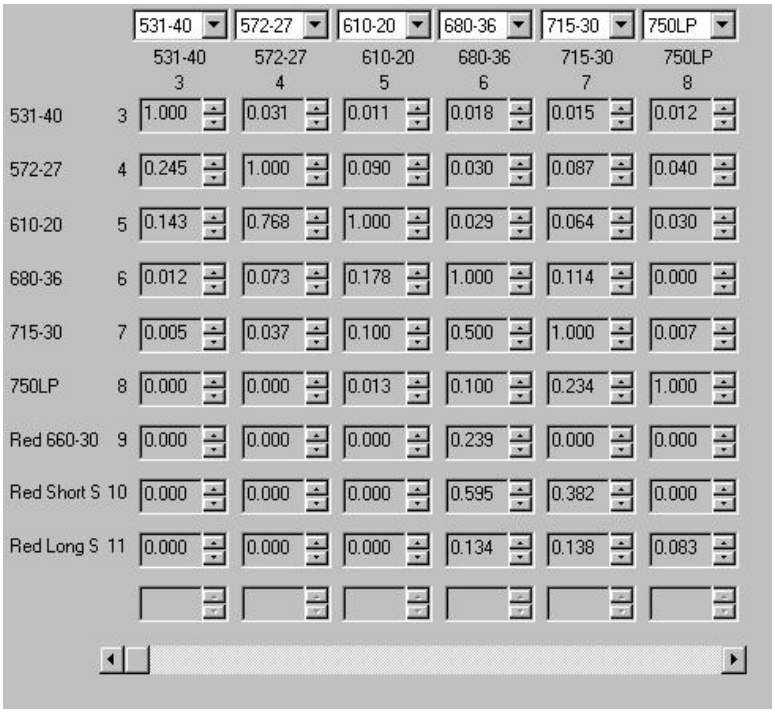
4.11.2 *Method 1: Use Spigot to Compensate in real-time*

Once PMT gains are set Spigot may be used to compensate data in real time. Use the following procedure for each fluorescent parameter to be compensated.

In Spigot's Left Display plot the first fluorescence parameter to be compensated vs. another fluorescence parameter. In the box next to the parameter drop down dialog click *Compensate* for both parameters so that compensated data will be plotted.

Compensation matrix values will be entered from Spigot's **COMP** menu. Open the **COMP** menu and choose all parameters to be compensated in the matrix. Load Unity Matrix may also be selected, in which case all active channels will be loaded into the matrix and parameters may be deleted (such as scatter) that will not be compensated.

Run the first sample to be compensated. Each sample should be positive for one channel only (ideally) so with this method that parameter will be plotted against all other parameters and will be compensated out of each negative parameter. In the following example matrix the 531-40 parameter is the first parameter to compensate out of all others. In this case the positive sample for the 531-40 fluorescence parameter should be run first.



Working down the column, plot the 531-40 channel vs. all successive channels. For example, start by plotting 531-40 vs. 572-27. Making sure that the *Compensate* box is checked in for each channel, begin increasing the matrix value in the first column, row 2 (531-40 vs. 572-27). Watch the data in the Left Display as it is compensated, and continue to

increase or decrease the matrix value until the data is compensated out of the 572-27 parameter (all or most data below first decade). Continue plotting 531-40 vs. all negative parameters and working down the matrix column compensate the data out of each of the negative parameters.

Next run the next positive sample and use the procedure described above to compensate that parameter (the next column to the right, 572-27 in this example) out of all negative parameters.

Continue until all positive samples have been run and compensated out of all negative parameters.

Matrix values may be entered by clicking in the matrix and typing a value, or by using the arrow keys at the edge of each matrix entry.

4.11.3 *Method 2: Use Flowjo to Compensate offline*

The compensation matrix may be determined offline by saving single color *.fcs files and using Flowjo to analyze the data and compute the matrix. Follow instructions provided by Flowjo's online help to create a *.mtx (XML) compensation matrix file.

After creating and saving a *.mtx file in the file may be loaded into Spigot by clicking on ***Load Compensation Matrix*** in Spigot's **COMP** menu. Browse to the saved matrix file and load the compensation matrix. Matrix values may be altered after loading as described in method 1.

4.12 Drop Delay

Setting the proper drop delay is critical for sort purity and recovery.

When the same nozzle tip and sheath pressure are used each day the delay setting will be very similar day to day, and thus some of the following steps may not be necessary.

The following instructions describe how to find the proper delay setting without knowing the previous days setting.

It is very important that the fluid stream has had at least 30 minutes of warm up time to stabilize. It is also very important that there are no bubbles in the nozzle. Refer to the Stream Generation section to make sure that the nozzle is properly purged of bubbles.

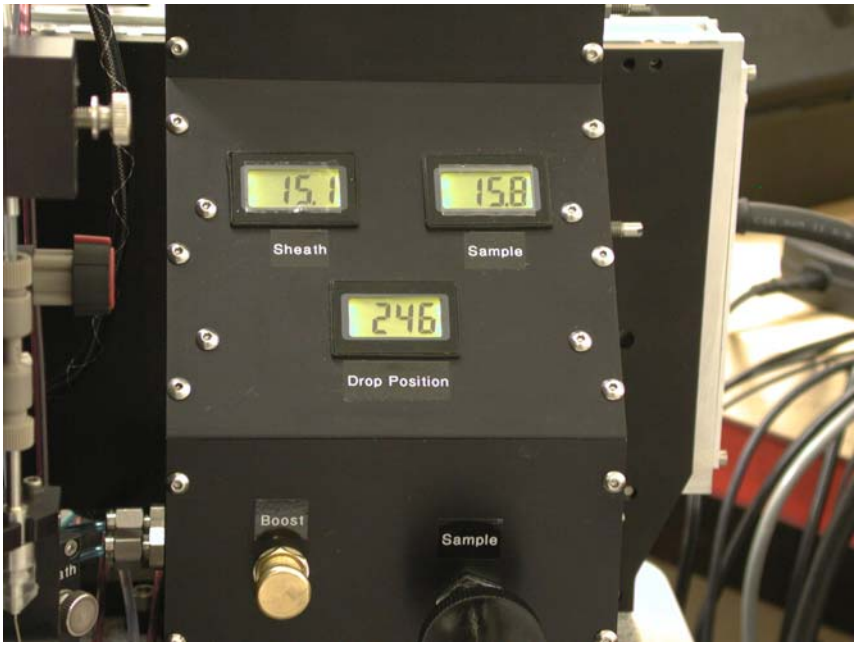
4.12.1 *Optimize droplet formation*

Re-optimize the BOP as described in the above section on Droplet Formation.

Use the **PIEZO AMPLITUDE** knob to ensure that the waveform of the jet at the BOP remains constant throughout the delay calibration process. Minor adjustments to the **PIEZO AMPLITUDE** knob are normal, if a major adjustment is necessary then check to make sure that there are no bubbles or clogs in the nozzle.

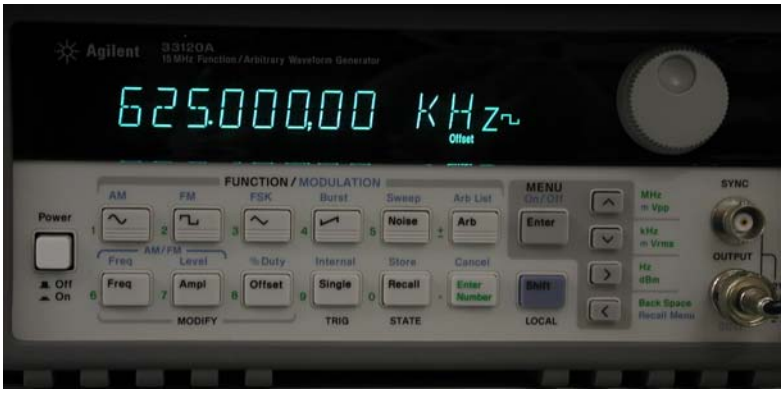
4.12.2 *Use Spigot to approximate Drop Delay*

Open the **SORT DELAY** screen in Spigot. Scan the drop camera to the points indicated, using the bottom or top edge of the drop monitor as a reference point. Enter the value of the *DROP POSITION* (indicated on the Pressure Console) for each position.



Drop Position readout on Pressure Console

Enter the master clock frequency *in Hz* and click *Calculate*.



Clock setting (KHz) on Function Generator

Spigot will calculate the approximate drop delay. Click *Set Drop Delay* in Spigot to set the system drop delay to the calculated value.

4.12.3 *Run test series with the WDU*

Run calibration particles at a low rate (100-1000/sec) and draw a SW around them as described in the Sort Gating section.

From the **SORT TRAY** screen in Spigot send the WDU to *Present*. Place a microscope slide in the WDU insert in the first position. Send the WDU to *Calibration*⇨*Sort Ready*.

Choose the number of particles to be sorted for the calibration run (defaults to 20). It is recommended (and these instructions will assume) that the sort count be set to 20.

Open the **SORT TRAY** screen in Spigot and click *Sort!.* The WDU will place a 5X5 matrix of puddles on the slide. Each puddle on the slide represents a sort command for the set number of particles (20) at different delay settings. After the WDU deposits the 25 puddles a screen will pop up that lists the delay setting Spigot used for each of the 25 puddles.

Take the slide to a fluorescent microscope and count the number of particles in each puddle. A Drop Delay Worksheet is provided in the appendix to this manual as a suggested method for keeping track of the particle counts per puddle. Identify the three puddles in series with the highest particle count. Take note of the position of the middle puddle of the series of three with the highest count. Read the delay value from the Spigot delay setting popup that corresponds to the puddle indicated above. This will be the delay setting used for the next calibration run. Set Drop Delay

Click on the proper delay value in the pop-up dialog and click *OK* to set the drop delay, alternatively the delay may be set from the **SIMPLE CONFIG** menu.

4.12.4 *Repeat Delay Calibration*

Repeat the above steps until two calibration series in a row yield the same value for the delay. This will be the delay setting used for the rest of the day. Remember that the sheath pressure, master clock setting, and BOP all need to remain constant in order for this delay setting to be accurate. If any of those settings change the delay will need to be reset as described above.

4.13 Sort Modes

When sorting, it is important to consider both coincident particles and the position of particles inside droplets. Coincidence occurs when two or more particles are closer than the spacing of the droplets, so that more than one particle ends up in the droplet. Obviously, these droplets must not be sorted in order to achieve maximum purity. Coincidence is higher when sample rates are higher and when droplet rates are lower; thus,

choosing a proper sort mode depends on those settings and the desired result of the sort: purity or recovery.

When the exact count of a sort is important, such as when sorting one particle into each of 96 wells, it is important to consider the position of the particle inside the droplet since particles near the droplet boundaries may end up in either droplet, and empty droplets may inadvertently be sorted.

The inFlux sorter provides circuitry to take care of these issues. Coincidence circuitry allows the user to set a coincidence window (in number of drops) before or after an event is detected. If another particle is detected within the coincidence window, the particles will not be sorted. A 1 ½ drop sort mode is also available. The 1 ½ drop sort mode will sort two drops when a particle is on a droplet boundary and one particle when a droplet is in the center of a drop, ensuring an accurate sort count.

Cytopeia recommends three different combinations of these two settings to arrive at useful sort modes depending on the desired sort outcome of the sort. Other settings may be experimented with as desired.

In this section we define:

Recovery as the number of particles sorted out of the number requested to be sorted.

Total recovery, or ***Yield***, is defined as number of desired particles sorted out of the total number of desired particles on the entire sample aliquot.

Setting 1: High Throughput, High Purity, High Yield, Lower Recovery

Use this setting when sample rates are high compared to droplet rates, and when the count (Recovery) of the sorted particles is less important.

Pre and Post Coincidence = 1 drop, 1 drop sort

Setting 2: Low Throughput, High Purity, Lower Yield, High Recovery

Use this setting when sample rates are low compared to droplet rates, and when the count of the sorted particles is important. This mode is most often used for single cell sorting into multi-well plates, and can also be useful when sorting large particles.

Pre and Post Coincidence = 1 ½ drop, 1 ½ drop sort

Setting 3: Low Throughput, Lower Purity, High Yield, High Recovery

Use this setting when high yield is desired and purity is not as important. The purity of a sort with coincidence disabled is completely determined by the sample to droplet rate ratio, and may be quite compromised at high sample rates. This mode is most often used for collecting rare populations where sort yield is more important than purity.

Pre and Post Coincidence = Disable Coincidence, 1 ½ drop sort

How to set the coincidence and sort mode:

The **1-1½** switch on the control panel switches the sort mode from 1-drop (switch down) to 1.5-drop (switch up) while sorting. Note that the same switch is also used to fine tune the phase of the BOP as described in the droplet formation section above. This function is unrelated to the sort mode function and is only intended to be used for set-up, and not for sorting.

Coincidence settings can be changed in the **CONFIGURE ADCs** menu in Spigot. Do not set the coincidence higher than 1 ½ drop.

4.14 Sample Collection

The WDU feature allows sample to be collected into 5 ml sample tubes and 96-well plates. Additional collection trays can be developed for special configurations.

Make sure that the system is aligned, droplet formation is stable, and that the delay setting has been calibrated before collecting sample.

4.14.1 *Running Samples*

When running samples it is critical that the sample line be back-flushed for about 30 seconds when switching to new samples to minimize sample carryover. If sample carryover is a major concern, load a sample tube with sheath fluid and run clean sheath through the sample line at a high sample pressure (~3 PSI over the sheath pressure – hold boost button) until no particles are detected. Alternatively, a new sample line can be installed with ease, ensuring no sample carryover.

4.14.2 *Left and/or Right Sort*

To collect up to two sample populations simultaneously, use the *2-Tube* sort mode. To ensure that the sort streams will fall into the collection tubes you may wish to double check the setting of the **PIEZO** and the **STREAM DEFLECTION** amplitude as described in the droplet formation section.

Load two 5 ml sample tubes into the WDU insert. Send the tray to the *2-Tube* ⇨ *Sort Ready* position. Make sure that the **PLATES** are on and that the **STREAM DEFLECTION** is on. Choose the number of cells to be sorted left or right from the **SORT** screen. Click on *Left Sort* or *Right Sort* to begin sorting (based on your gating scheme) into the collection tubes. A progress bar will pop up in Spigot that will give feedback on the number of cells sorted as the sort progresses, as well as an abort rate indicated near a circular red icon.

Use the monitors during sorting to ensure that particles are being sorted and that the BOP remains constant.

A sort may be paused or terminated from Spigot. When the sort is done

the WDU will place the tray in the *Safe* position. The tray can then be presented and sample tubes can be collected.

4.14.3 *96-Well Sort*

96-well sorting allows a particular number of particles to be placed into each of the wells of a 96 well plate. Follow directions above but use the *96-Well* mode for all tray settings. Remember that the sort ready position may be offset to ensure that the sort streams will fall into the center of the wells.

4.14.4 *Sort Bypass*

Selecting *Bypass* mode in the right toolbar's sort controls, rather than *Normal*, will enable sorting without counting. As much sorted sample as available in the sample tube will be sorted without count feedback.

4.15 Shutting Down

4.15.1 *Close Spigot*

4.15.2 *Shut down Fluidics*

For optimal results and decreased chances of contamination, the inFlux fluidics should be shut down, emptied out and allowed to dry overnight. Use the following shutdown procedure:

Allow sample line to back flush for at least 1 minute.

Close all fluid valves by clicking RUN off.

Turn off sheath pressure by flipping the **AIR** switch down on the Pressure Console.

Empty sheath reservoir, rinse and fill with about 200 mL of 0.22mm filtered distilled or de-ionized water.

Reattach sheath reservoir and flip up **AIR** switch on pressure console.

Click RINSE and BACKFLUSH to let the clean water flow through the system until it has emptied the sheath reservoir and the system blows air. Allow the system to blow air until no more water is coming up the sheath line and until no more water drips from the sample line.

Click RUN off, remove nozzle tip (put nozzle tip in a safe place), place flush bucket under nozzle assembly. Click RINSE on and let system blow air for 10-15 minutes to fully dry out tubing.

Flip the **AIR** switch off on the pressure console.

Remove sheath and waste reservoir and empty all liquids. Rinse and or clean reservoirs as necessary.

Turn reservoirs upside down on the table on a piece of absorbing tissue. Be careful not to damage gauges or fittings.

4.15.3 *Shut Down Control Panel Switches*

Switch all switches on the control panel to the off position.

4.15.4 *Clean Sort Chamber*

Loosen Deflection Plate thumbscrews to open and clean plates. Use a wet wipe to clean away any salt build up on the drain or in the sort chamber.

4.15.5 *Shut down Electronics Console*

Turn the Electronics Console off by flipping the **MAIN POWER** switch on the bottom left of the console to the **OFF** position. Turn off all switches on the Control Panel.

4.15.6 *Shut down Lasers*

Shut down all lasers as specified by the manufacturer.

[End of Chapter]

Chapter 5 - Spigot Interface

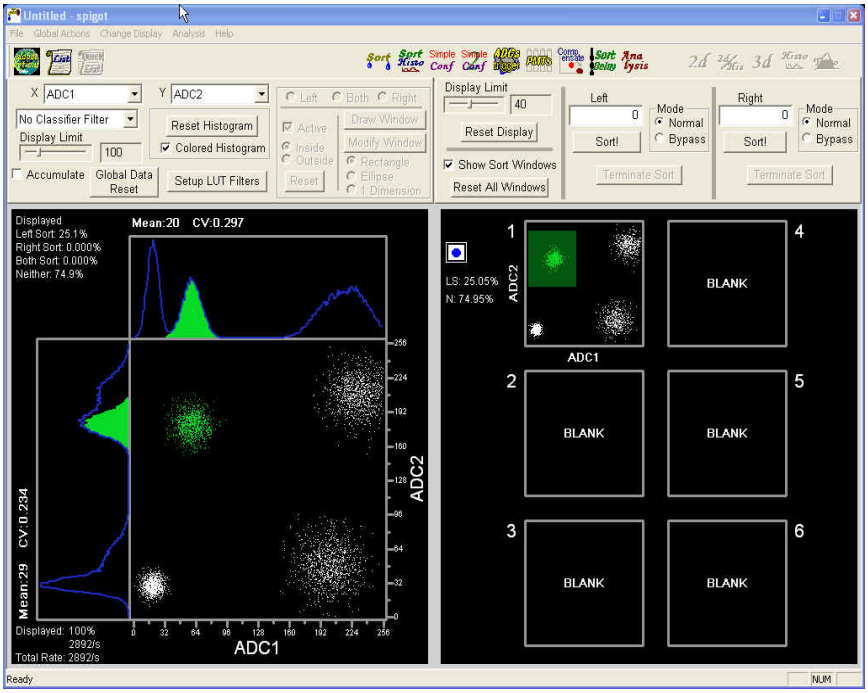
Summary: *This chapter describes the layout of the Spigot interface and the various options available to the user within each of the various screens.*

5.1 Overview

The Spigot interface consists of three main components: the toolbars and the left and right displays (see below). The size and position of these three components on the screen cannot be changed.

The left display is fixed in place, and it always contains a two-dimensional (2D) data display with corresponding histograms. Particle rate, sort percentages and other data are displayed here as well.

The right display can show a variety of configuration and data analysis screens.



Spigot Display

5.2 Left Display

The left display shows a 2D, real-time dot plot. Each dot represents data the sorter has received from its photomultiplier tubes (PMTs) as processed by the analog-to-digital converters (ADCs). This data represent the intensity, as detected by the PMTs, of light re-emitted by particles in the sample stream upon laser excitation, after the light has passed

through the instrument's filter set.

5.2.1 *Basic information*

The user can select which parameters to display on the X and Y axes of the left display by choosing from available parameters from the drop-down lists labeled *X* and *Y* on the left side of the toolbar. Particle injection rates are shown in the lower left hand corner. A particle counter is available by left-clicking the rate region or using the keyboard shortcut **CTRL-SPACE** and functions similar to a stopwatch. The default color of the dots displayed in the 2D plot is white, and they change color depending on whether they fall in gates corresponding to the left (green), right (red), or both (yellow) sort directions. The user has the option of changing these colors using **GLOBAL OPTIONS**, discussed later in this chapter.

5.2.2 *Sort windows*

For sorting operations, the left display is used in conjunction with the right display's sort screen for drawing sort windows (SWs). When one of the six LUTs displayed on the right display is highlighted with an orange border, the left display changes to show the same parameters as the highlighted LUT. SWs can be drawn to any shape, moved, resized, or removed in the left display.

5.2.3 *Classifier filters*

Selecting from the *Classifier Filter* drop-down list on the left side of the toolbar allows the user to see what is actually being sorted based on the SWs drawn and activated in the LUTs. For example, applying a *Left Classifier Filter* displays the result of all the active left SWs drawn in the LUTs (using "and" logic). Applying these filters does not alter the actual sort. Sort decisions are governed by the SWs as drawn and activated using the right display's LUTs; applying a classifier filter only modifies the displayed data.

5.2.4 *LUT filters*

For additional convenience during sorting, the user can simulate the effect of individual LUTs by selecting *Show LUT Filters* in the left side of the toolbar. This places six small blue squares, representative of the six LUTs, in the upper left corner of the left display's dot plot. By clicking on one or more of these squares, the user can simulate how the dot plot and sort results would change if particular LUTs were made active or inactive. Thus, if six LUTs have been defined, this LUT simulation tool can be used to evaluate the pass-through (or exclusion) of particles by the LUTs, either individually or in groups. This allows displaying data using "or" logic, if desired. Again, applying a LUT filter only changes the displayed data, not the actual particles being sorted.

5.2.5 *Sort data*

Percentages of particles displayed as falling into the left and right SWs

are displayed in the upper left hand corner of the left display. Clicking on that portion of the screen toggles the displayed data between percentages and particles/sec. This data will change with application of classifier filters.

5.2.6 *Histograms*

The histogram is also correspondingly colored so that the user can quickly recognize how the distribution of points falls within each sort gate. Peak and coefficient of variation information for each histogram is also displayed. By moving the mouse pointer over the dot plot, the user can move cross-hairs to determine the coordinates of any particular point on the plot.

5.2.7 *Quadrant data*

If the user clicks the left mouse button while positioning these cross-hairs, blue lines will be placed in the plot dividing it into quadrants. The numbers of particles falling within each quadrant is displayed in small blue boxes in the upper left area of the display.

5.3 Right Display

The right display can be customized to show a number of different instrument configuration and data analysis screens. The available screens are:

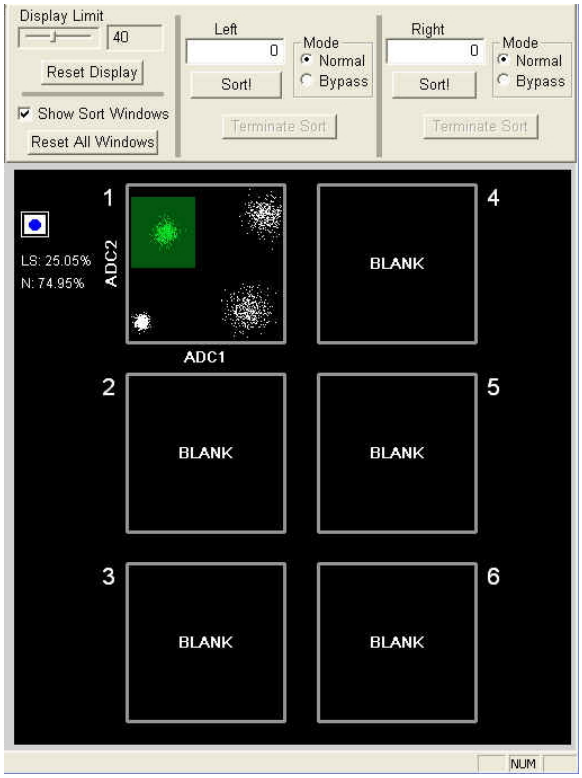
- Sort Controls
- Sort Controls with Histograms
- Simple Hardware Configuration (“Simple Config”)
- Simple Hardware Configuration 2 (“Simple Config 2”)
- Configure ADCs
- Configure PMTs
- Configure Compensation
- Sort Settings
- Analysis (with five different views)
- Sort Tray

The right display screens can be selected by clicking on one of the colored buttons on the toolbar or via the **CHANGE DISPLAY** drop down menu on the menu bar. Some of the screens present overlapping data or configuration choices. Users can set the same parameter on more than one screen and other affected screens are immediately updated to reflect the new setting. Where appropriate the right main section of the toolbar changes to allow user input for customizing the selected screen and setting various parameters. In other cases the right section of the toolbar is completely replaced by the selected screen.

Each of these right display screens are described in more detail in the remainder of this section.

5.3.1 *Sort Controls*

When Spigot is first started, or if **SORT** is clicked, the right display shows six numbered squares representing LUTs, and the right side of the toolbar changes to show sorting related options. For example, the figure at right shows LUT 1 with a left (green) SW active.



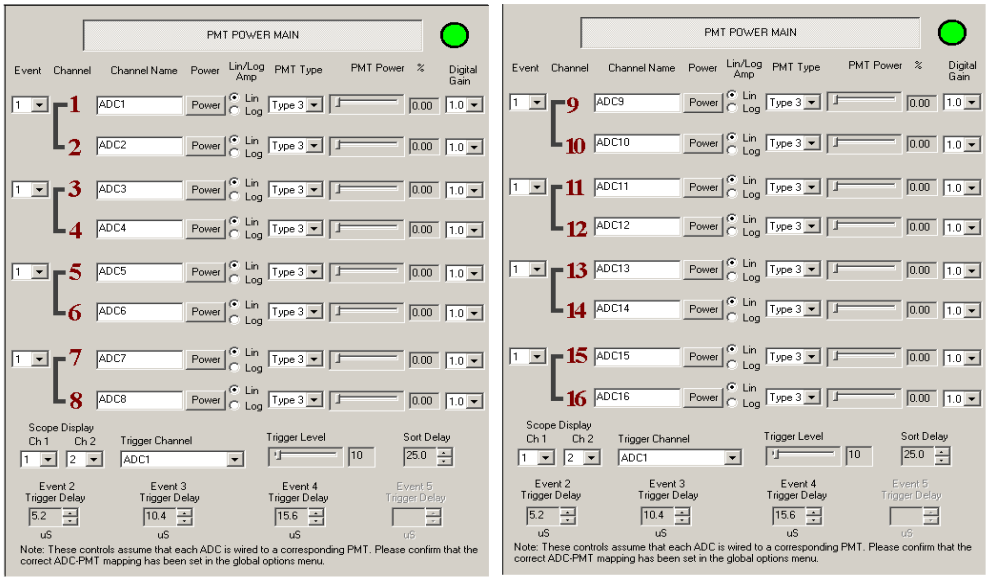
Sort Controls

Spigot thus enables specifying up to twelve SWs (six left and six right). For each set, the current data is shown relative to the axes in which the SWs are defined. These smaller displays are updated in real-time similar to the larger dot plot in the left display, and immediately reflect changes made in the SW via the left display. Dots are also color-coded in these windows in similar fashion to the left window.

Clicking on any LUT immediately brings its contents up on the left display. In this way, users can watch their data in real-time on up to six sets of axes and quickly switch between these axes on the larger left display. Statistics are also displayed for each of the defined SWs as well as current SW status information. Once the SWs have been created to the user's satisfaction, sort controls are conveniently located at the top of the screen.

5.3.2 *Simple Hardware Configuration*

SIMPLE CONFIG is a right display screen (below left) that shows a comprehensive collection of options to configure the Spigot interface for ADC channels 1 through 8. This display includes some of the commonly used settings available via the Configure ADCs and Configure PMTs screens, as well as some **GLOBAL OPTIONS**. The **PMT POWER MAIN** button will power off and on all PMTs.



Simple Config screens

5.3.3 *Simple Hardware Configuration 2*

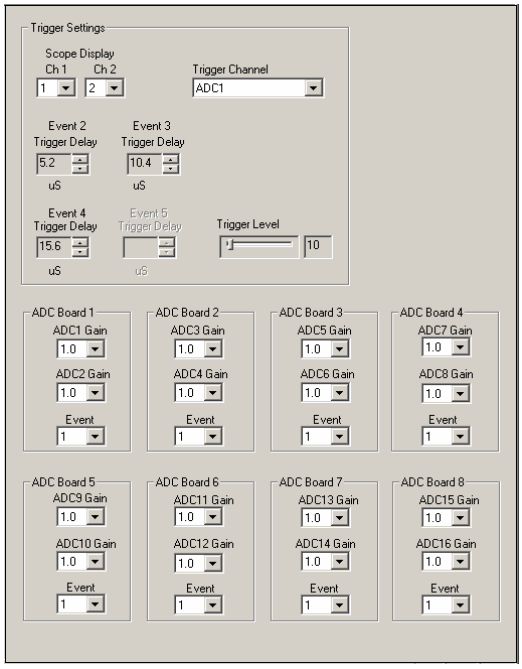
The **SIMPLE CONFIG 2** screen (above right) is identical to the **SIMPLE CONFIG** display, except that more channels may be configured, up to a total of 16, depending on the number of ADCs that are installed on the inFlux. **SIMPLE CONFIG 2** shows channels 9 through 16, while **SIMPLE CONFIG** shows channels 1 through 8.

5.3.4 *Configure ADCs*

This screen (shown at right) allows user configuration of the ADCs. All of these settings are available from the hardware configuration screens.

On the bottom of the screen, each ADC's digital gain can be adjusted, and ADCs can be assigned to one of three "events."

On the upper left side of the screen – *Trigger Settings* -- ADC channels are selected for display on the oscilloscope using the *Scope Display* control. Below that are is a slider control for setting *Trigger Level* for Event 1and spin controls for setting the *Trigger Delay* for Events 2 through 5, if available.



5.3.5 *Configure PMTs*

Each of the PMTs installed on the inFlux may be individually configured using this right display screen (Shown at right). All of these controls are also available on the hardware configuration screens. The **PMT POWER MAIN** button will power off and on all PMTs.

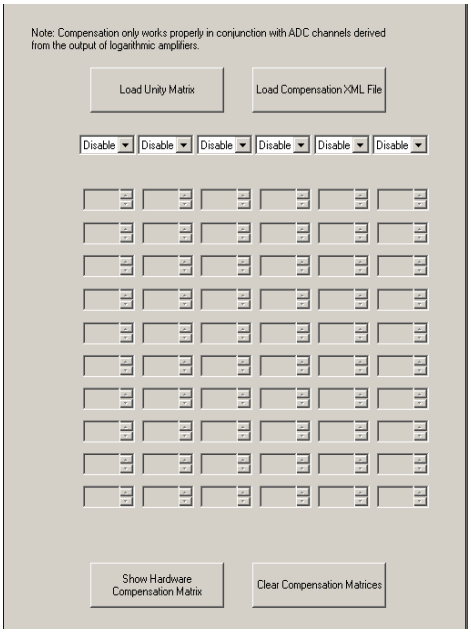
For each PMT, power can be toggled on and off, and a green light shows power on. Gain may be adjusted using the slider control. (Note: Gain can be adjusted using orange triangular sliders on axes of the 2D plots if enabled in **GLOBAL OPTIONS**.)

PMT amplification may also be toggled between logarithmic and linear. The type of each PMT installed on the inFlux may be set using the drop-down list.

5.3.6 *Configure Compensation*

Spigot can be configured to change the data display to compensate for spectral overlap. Clicking **COMPENSATE** changes the right display to that shown at right.

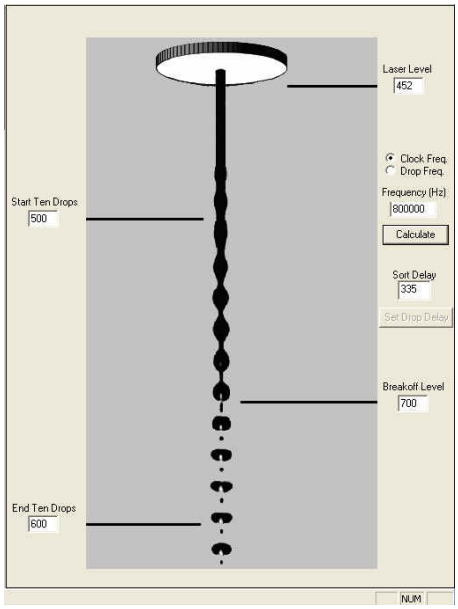
Compensation values can be loaded and edited in this window. The Unity Matrix is an identity matrix with all active ADC channels. You can also load compensation files from an XML format.



5.3.7 *Sort Delay*

Spigot assists the inFlux operator by providing a convenient method for calculating the sort delay setting. Selecting **SORT DELAY** places a graphical representation of the sort stream in the right display.

There are windows for entering drop camera values corresponding to four points on the stream, and for entering clock or drop frequency. Spigot estimates the sort delay setting based on these inputs when the *Calculate* button is clicked.

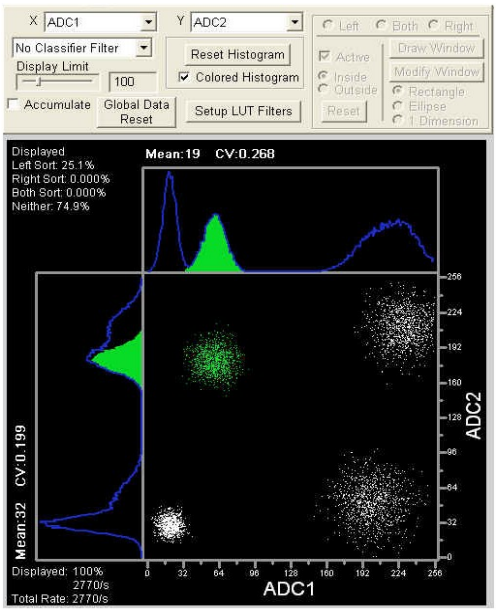


Sort Delay

5.3.8 *Analysis*

The right display can be configured to display five different analysis screens by using the analysis buttons on the button bar. The available screens are:

2D/Histogram
2D Plot
3DPlot
Histograms
Topographic Histogram.



2D/Histogram

Buttons for these views are inactive until **ANALYSIS** is clicked.

The first time **ANALYSIS** is selected after starting the Spigot application, the default view is *2D/Histogram* (right). The user can then navigate through the five different views by clicking the now active analysis buttons. If the user selects a right display option other than one of the analysis views, the analysis buttons once again become inactive. However, Spigot remembers the analysis view last used, so that subsequent clicking on **ANALYSIS** brings up the most recently viewed screen option.

The right section of the toolbar also changes with each different analysis view selection, giving users many options for customizing the data display.

In each of the analysis views, the data points or histograms change color depending on the SWs drawn. Whenever SWs are drawn or modified, the analysis views are updated simultaneously.

5.3.9 *2D/Histogram View*

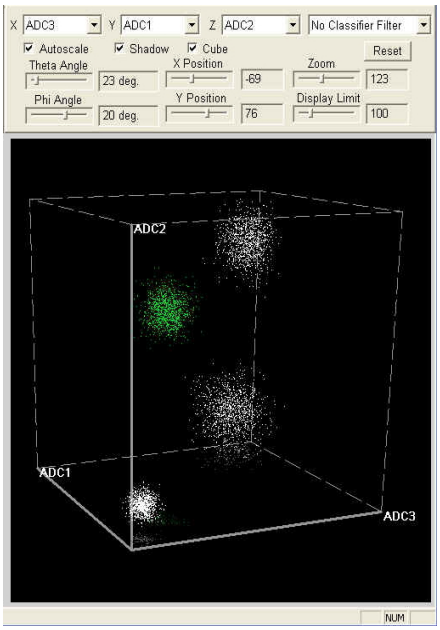
This default analysis view is similar to the left display, except that SWs cannot be drawn here.

5.3.10 *2D Plot View* 

The *2D* analysis screen is simply the *2D/His* screen without the histogram and additional data display. It provides a simplified, uncluttered view of the dot plot.

5.3.11 *3D Plot View* 

The *3D* analysis view adds the additional feature of visualizing the data in a three axes perspective dot plot (see right).



3D Plot

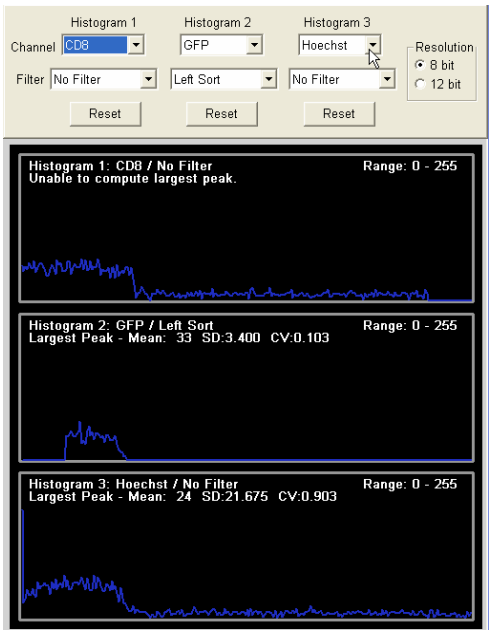
In this view, the right side of the toolbar enables selection of the three parameters displayed, as well as the application of a classifier filter. The theta and phi angles of the display can also be changed with a slider control. As with most displays in Spigot, the limit of dots displayed can be adjusted using the *Display Limit* slider control.

If *Autoscale* is unchecked, the plot can also be moved in the X and Y directions, and can be zoomed in and out, using slider controls located in the toolbar above the display.

If the *Shadow* box is checked, Spigot draws a colored dot “shadow” on the lower plane of the display.

If the *Cube* box is checked, Spigot draws dashed lines to complete a cube, allowing another way to visualize the data in 3D.

5.3.12 *Histograms View*



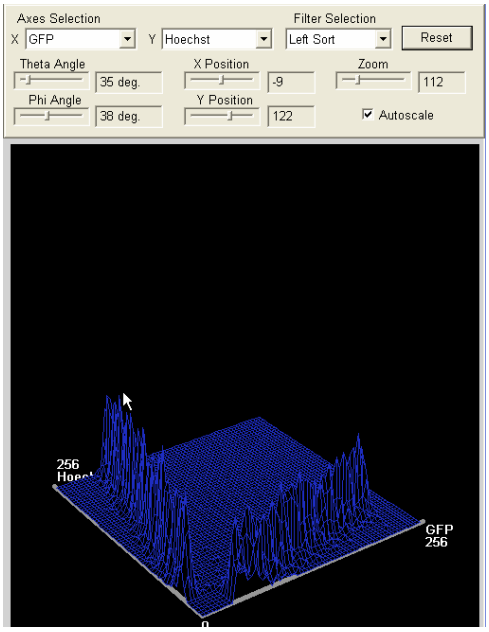
Histograms

In the *Histogram* view (shown at right), three histograms allow convenient comparison of data from three channels.

The right side of the toolbar has drop-down lists for selecting a channel for each histogram to display, and for applying any classifier filter desired. *Reset* buttons allow resetting of individual histograms.

There is also an option to display the data in 8-bit or 12-bit resolution. *Note: This feature changes only the display resolution; the inFlux stores data at 12-bit resolution*.

5.3.13 *Topographic Histogram View*



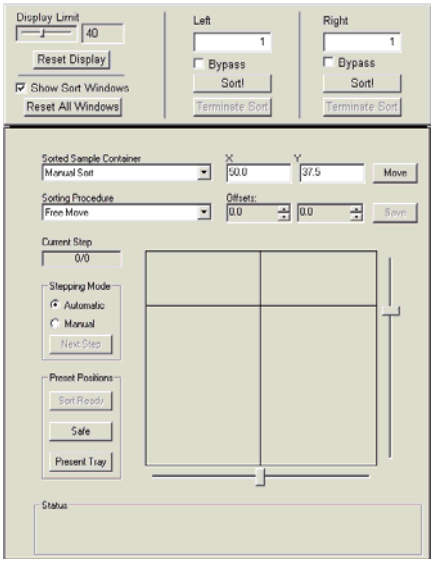
Topo Histogram

This analysis view presents the data in a 3D topographic histogram (shown at right). The right side of the toolbar contains drop-down lists for selecting the channels to be displayed on the X and Y axes of the histogram. A classifier filter may be applied.

The *Reset* button resets the histogram, and there is a checkbox for changing between “rising” and “continuous” display modes.

Orientation of the display can be adjusted using the *Theta Angle*, *Phi Angle*, *X Position* and *Y Position* slider controls on the toolbar (numerical values are shown for reference). These controls affect the display differently depending on the *Autoscale* selection. If *Autoscale* is unchecked, the display can be zoomed using the *Zoom* slider control.

5.3.14 *Sort Tray* 



Software control of the WDU is accessed by selecting **SORT TRAY** from the button bar.

The right toolbar section continues to display sort controls so that it is possible to initiate a sort from the screen.

The **TRAY** screen contains two sliders, in-out (y-axis) and left-right (x-axis), that allow the tray to be moved to any position within its range of motion. The numbers displayed in the upper-right portion of the lower dialog box represent the current position of the WDU tray in millimeters from the home position (fully in, fully right).

The top left of the lower dialog has drop-down lists to select the type of tray you will be sorting your samples into. Currently defined are 96 well, two-tube, calibration slide, and a manual control so you can move the WDU tray freely. Below this drop-down list is the sorting procedure, which is how the selected tray moves through the various positions of its wells, if applicable. The offsets made in the two controls to the right of the sorting procedure allow you to give a little nudge to the WDU position in the event that it's not quite lined up properly. These offsets can be stored in your tray settings by using the save button to the right of them.

Below the sorting procedure list box is a display of the current step out of all of the steps defined in the sorting procedure.

Below the step listing is an auto/manual control, which, if set to manual, halts the sort after the count of particles has been sorted until you click the next step button.

Below the stepping mode control is a couple of presets. **SORT READY** moves the tray to the first position in your sorting procedure. **SAFE** moves the tray to a position where your samples should not get contaminated by the sorting stream. **PRESENT TRAY** moves your sample tray front-and-center so you may remove your sorted samples from the WDU.

5.4 Toolbar

Note: While the foregoing introduction to the various right display screens already has covered most of the options available to the user from the toolbars, this section presents information on additional toolbar options and features.

The toolbar at the top of the screen consist of two main toolbar sections

above the right and left displays, a button bar for selecting common functions and tasks, and a typical menu bar at the top of the screen.

Left Main Toolbar

5.4.1 *Toolbars*

The left main toolbar section does not change, except that certain options will be displayed as unavailable depending on other choices the user has made. For example, if a SW has not been highlighted by the user, the toolbar section for drawing a SW will not be activated.

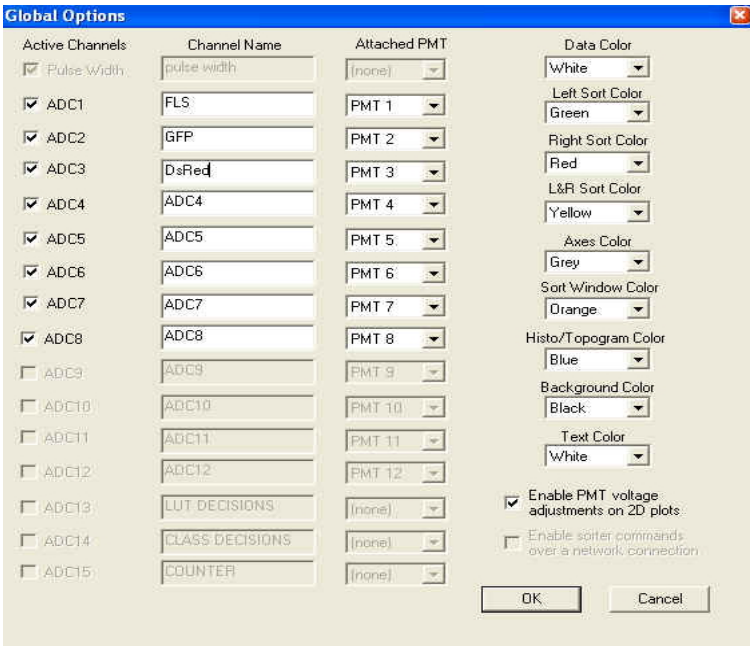
The right main toolbar section often changes with changes in the right display and, in some cases, is completely replaced by the right display.

5.4.2 *Button Bar*

The button bar has labeled, color buttons for selecting each of the available right display screens. These have already been described above. On the left side of the button bar are three additional buttons: **GLOBAL OPTIONS**, **LIST** and **QUICK LIST**.

Global Options 

Spigot may be also customized to fit user preference and inFlux configuration by clicking on **GLOBAL OPTIONS** or by selecting **GLOBAL ACTIONS: GLOBAL OPTIONS** from the Global Actions drop down list on the menu bar. The dialog box that appears is shown below.



Global Options

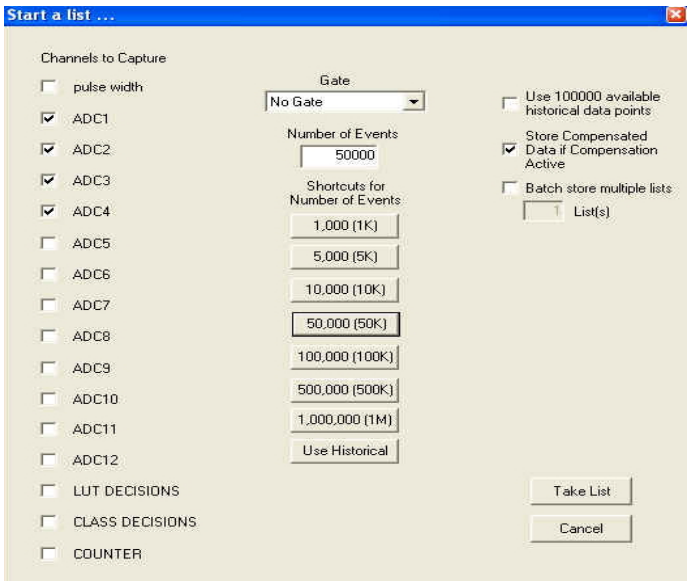
Individual ADC channels may be deactivated by clearing the check boxes on the left side of the dialog box. Next, channel names may be changed from the default names ADC1, ADC2 etc. (this feature is also available on the hardware configuration screens.) On the right side of the dialog box, users may choose from among several colors on drop down lists for customizing the data displays. There is also a check box for toggling the slider type PMT gain control on the 2D dot plots displays.

The **GLOBAL OPTIONS** tool also allows “remapping” of PMTs to ADCs from the default configuration of ADC1 to PMT1, ADC2 to PMT2, and so on. Each drop-down list under *Attached PMT* enables mapping any PMT to a particular ADC, to reflect a change in hardware wiring of ADCs and PMTs.

CAUTION	This feature should only be used by advanced users or technicians as it depends on the instrument’s hardware configuration. Spigot and the inFlux will not operate properly if this feature is set up incorrectly.

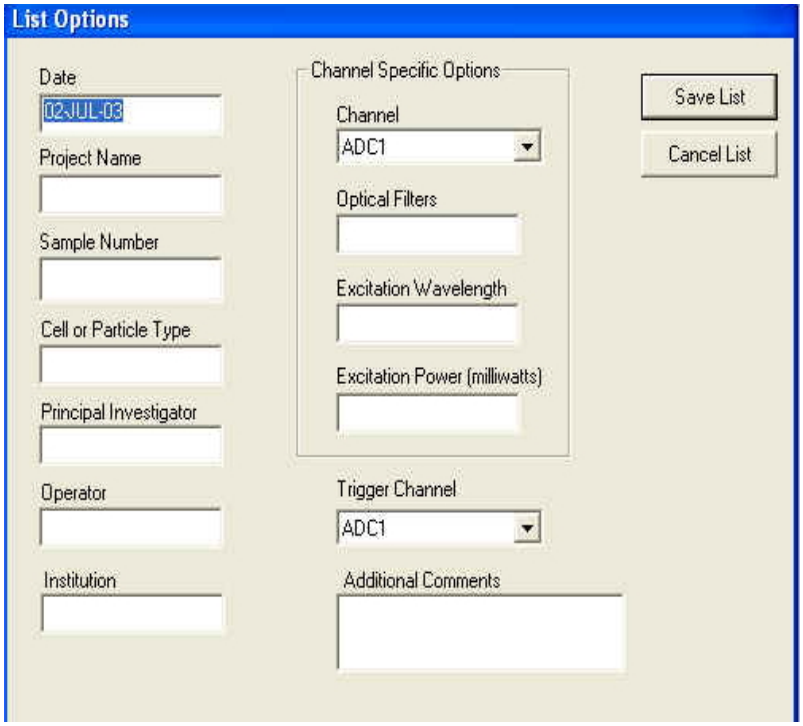
List Tool 

Spigot has the ability to capture data and store it in files using the standard flow cytometry format (*.fcs). This data capture is configured using the List Tool, available by clicking **LIST** (or by selecting **GLOBAL ACTIONS: TAKE A LIST** from the drop down list on the menu bar). In the *Start a List* dialog box that appears (shown below) specific channels and parameters may be checked for inclusion in the data capture. The number of events to be captured may be set in the center of the dialog box, either by typing numbers in the window or by clicking on one of the shortcut buttons placed under the window for user convenience.



Start a List

After setting these list parameters, clicking *Take List* starts the data capture. A *List Options* dialog box (shown below) also appears for attaching reference information to the file being created. Clicking on *Save List* brings up a standard Windows “Save As” dialog box for naming and saving the new list in a preferred location.



List Options

Quick List

Once data capture has been configured using **LIST**, the user can repeatedly save data to files using the same list settings by utilizing the **QUICK LIST** feature. The **QUICK LIST** button (and **GLOBAL OPTIONS: QUICK LIST** from the drop down list on the menu bar) is active when the List Tool has been configured. Clicking on **QUICK LIST** starts a new data capture and saves the data to a new .fcs file with a sequential number appended to the file name. For example, if the **LIST** was initially configured to save to a file named test.fcs, then subsequent files saved using **QUICK LIST** would be test1.fcs, test2.fcs, etc., all saved to the same directory path as the first file.

5.4.3 *Menu Options*

Under **FILE**, the user can open an existing Spigot Configuration File (*.sco) or save a current one to be used later. There are also options for printing the left or right displays, or both.

The **GLOBAL ACTIONS** menu contains selections for displaying or store the raw data stream from the sorter.

In all other cases, the options available from the menu bar duplicate

options available simply by clicking on buttons.

[End of Chapter]

Chapter 6 - Maintenance

Summary: *This chapter covers recommended maintenance of the inFlux and provides operators with contact information for customer support at Cytopeia if technical assistance is needed.*

6.1 Maintenance

6.1.1 *Periodic User Cleaning and Inspection*

Daily

After each day's operation, the fluidics system should be cleared and dried out thoroughly using the following procedure:

1. Turn air switch to off
2. Release sheath reservoir pressure, then disconnect, drain and rinse reservoir.
3. Refill sheath reservoir with 100 mL of de-ionized water, reconnect sheath reservoir and turn air switch on.
4. Run this fluid through entire system, including backflushing the sample line, until the reservoir runs dry.
5. Continue to run air through system for approximately 15 minutes, ensuring that all lines are free of fluids.
6. During this time, the nozzle tip may be removed, sonicated if desired, and left to dry in a protected area.
7. Shut down fluidics by turning off air switch and vacuum pump.
8. Empty, rinse and air dry both reservoirs.

Weekly

To keep the inFlux running well for a long time, perform the following on at least weekly basis:

1. Remove dust from all exposed surfaces.
2. Clean salt buildups from the parts and areas exposed to sheath or sample fluids.
3. Clean the WDU tray.
4. Vacuum dust and lint from fan areas, such as the back of the sort electronics console, laser power supplies, and the computer.
5. Inspect tubing and fluidics for leaks.

6.1.2 *Operator Maintenance*

Fluidics: The inFlux has been designed so that all fluidics lines are replaceable by the user. To order replacement assemblies, and for guidance on installing these parts, please contact Cytopeia Customer Service. The inFlux system has been tested and validated using specific fluidics tubing. The use of any tubing other than that provided by or specified by Cytopeia may result in substandard instrument performance, damaging leakage or instrument failure, and will not be supported by Cytopeia.

Optics: The inFlux has been designed so that filters in the detector modules may be removed or interchanged by the user. Please contact Cytopeia Customer Service for guidance on this process and to order additional or replacement filters.

6.1.3 *Other Maintenance*

Any other maintenance should not be considered routine and should only be performed by Cytopeia service technicians. Please contact Cytopeia for service arrangements using the contact information below.

6.2 Support Information

In the event of difficulty with operating the inFlux or to report a malfunctioning instrument or component, please send an email to:

support@cytopeia.com.

If immediate assistance is required, please call us at: 206-364-3400.

In the near future, we hope to have a technical support area on our website and we will notify you when it comes on line.

The mailing address for technical support is:

Cytopeia, Inc.
Attn: Customer Service
12730 28th Ave NE
Seattle, WA 98125

[End of chapter]

Appendix A – Drop Delay Worksheet

PSI: __________ B.O.P: __________
Clock:__________ Piezo: __________

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Delay Setting: _______

Instrument: ________________ Operator: _____________ Date: __________

Appendix B: Pressure / Frequency Chart

Use the following chart as a guideline for approximate available Pressure / Frequency settings. Actual Master Clock settings may be slightly different for each nozzle assembly, scan frequencies near recommended values to find the frequency with the shortest break off point. Recall that the actual drop rate is 1/16 of the master clock setting, and has been displayed below for convenience.

Tip Size	Pressure (PSI)	Mclock (Hz)	Drop Rate (HZ)
50	10	860000	53750
50	13	988000	61750
50	14.5	1088000	68000
50	17	1180000	73750
50	18.5	1261000	78813
50	22	1405000	87813
50	25	1500000	93750
50	27.5	1618000	101125
50	33	1788000	111750
50	41	2020000	126250
50	44	2093000	130813
50	47	2200000	137500
50	59	2513000	157063
50	72	2775000	173438
50	76	2881000	180063
50	85	3080000	192500
70	7	459000	28688
70	12.3	628000	39250
70	18.5	787000	49188
70	22	860000	53750
70	27.5	988000	61750
70	33	1088000	68000
70	39.3	1187000	74188
70	45	1261000	78813
70	52	1405000	87813
100	5.2	240000	15000
100	8.5	318000	19875
100	10.2	362000	22625
100	15	437000	27313
150	4.5	160000	10000