APOGEE FLOW SYSTEMS

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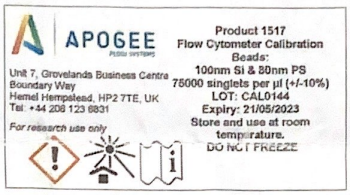
PRODUCT DATASHEET:
Flow Cytometer Calibration Beads
Cat#1517

Product Label:



Intended Use

A mixture of non fluorescent Silica (100nm) and fluorescent Latex (80nm) micro-spheres suspended in an aqueous detergent solution, prepared to a precise concentration, with precise light scatter and fluorescence characteristics as required for calibration of flow cytometers. For research use only.

Approximate particle concentrations for Lot # CAL0144:

Particle Size (nm)	Approximate number per microlitre
80nm	25 000
100nm	50 000

Storage Conditions

The calibration beads are stable until the expiration date shown on the label when stored at room temperature. Do not freeze. Do not expose to direct light during storage.

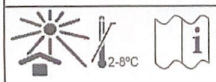
Instructions for Use

Please refer to the flow cytometer's Operator Manual. Use undiluted.

Warnings and Recommendations

1. Suitable for calibration of Apogee flow cytometers. Not for diagnostic use.
2. Product is stable until the expiration date shown on the label if it is properly stored. Do not use after the expiration date shown on the label. If the product is stored in conditions different from those recommended, such conditions must be validated by the user.
3. The detergent solution contains 0.1% sodium azide (CAS-Nr. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination or incorrect results may occur.
4. Sodium azide (NaN3) is harmful if swallowed (R22). If swallowed seek medical advice immediately and show the container or label (S46).
5. It is advisable to allow the calibration beads to warm to room temperature before running on the flow cytometer.

Explanation of Symbols

Symbol	Meaning
	Protect from light, Storage Temp Range, Consult Product User Manual Before Use
	Harmful substance (MSDS available)

Warranty

This product is warranted only to confirm to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Apogee Flow System's sole liability is limited to either replacement of the product or refund of the purchase price.

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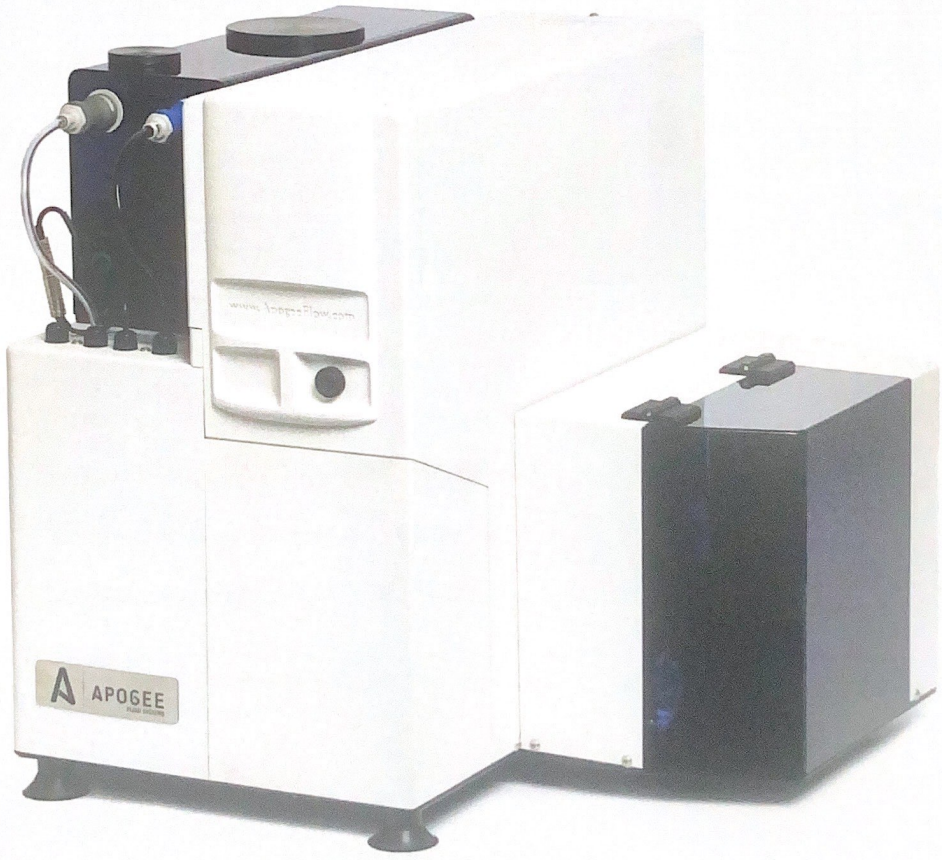
Operator Manual for 'Micro-PLUS', 'Micro' and 'Universal' Model Flow Cytometers

(A40, A50 and A60 Generations)

(Document Cat # 9919)



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Revision History

Revision	Date	Description	CO#	Authorized by
2.02	13 Apr 2010			
2.10	26 Jan 2011			
2.15	29 Jan 2013			
2.20	10 Nov 2014	Autosampler functionality and fault diagnosis update		OJK
2.21	15 Feb 2016	Added this revision table. Updated to include new A60 generation.	CO417	OJK
2.22	10 Jan 2017	Minor edits to the Batch Excel Export text		OJK
2.23	29 Mar 2017	Software configuration for networked machines and multiple Windows users (Section 6.2). General tidy of old information.		OJK
2.24	28 June 2017	Added quality control guidelines & section 4.3 on event rates & added a fluorescence compensation procedure		OJK
2.25	10 Aug 2017	Reduced recommended concentration of sheath fluid preservative from 500ul/tank to 200µl/tank (page 16). Amended section 7.1 to introduce section on data integrity: $ORIGINALITY and $OP FCS keywords.	CO471	OJK
2.26	7 Sept 2017	Added images of fluidics to assist fault diagnosis		OJK
2.27	16 Oct 2017	Improved the software installation instructions for multiple Windows users		OJK
2.28	25 Oct 2017	Added section on use of Terminal program to soak flow cell in cleaning fluid (11.3). Modified the Shutdown procedure in section 8.4.		OJK
2.29	15th Dec 2017	Edited chapters 10 & 11, trouble shooting and maintenance		OJK
2.30	23 Jan 2018	Added sample probe height information (Section 9.1)		OJK
2.31	07 Mar 2018	Added plate view feature for Autosampler and Flow Cell Soak button		OJK
2.32	19 Mar 2018	Minor edits to section 11.5: Sheath filter wetting		OJK
2.33	28 Mar 2018	Added image of sheath and cleaning fluid filters		OJK
2.34	29 Apr 2018	Developed the 21CFR Part 11 description, section 7.1. Improved information on the ROI statistics and added geometric mean option.	CO495	OJK
2.35	12/11/2018	Title change to explicitly include Micro-PLUS model		OJK
2.36	29 Nov 2018	Added laser classification note in section 1.3		OJK
2.37	18 Dec 2018	Added Protocol option in WorkList and description of path to the protocols folder		OJK
2.38	06 Jun 2019	Deleted some old material from Auto40 days. Added basic notes on GxP operation. Replaced 'PC Control' with 'FCM Control'		OJK
2.39	12 Jun 2019	Developed the use of Windows Task Scheduler		OJK
2.40	11 July 2019	Updated method to run as admin in section 7.1.1		OJK
2.41	21 Nov 2019	Added to the description of the ApoCAL option (section 7.1)		OJK
2.42	13Mar2020	Clarified filter wetting procedure and edited syringe quality test		OJK
2.43	20Apr2020	Added packing instructions (SA030)		OJK

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CONTENTS

1. Apogee Flow Cytometers

1.1 Introduction

This manual covers the general operating procedures and introduces some of the instrument's features and unique characteristics. For help with specific applications, it is recommended to follow the reagent manufacturer's guidelines and one of the many books available on flow cytometry.

Upon delivery/installation, you may be provided with additional operating notes and instructions for your specific version of machine.

1.2 Intended Use

Apogee Flow Cytometers contain a sophisticated combination of optical, electronic, fluid and software technologies. This operator manual covers the A40, A50 and A60 generations of the Micro and Universal models. Since its incorporation, Apogee has manufactured a range of models including:

- Micro-PLUS model (A50 or A60 generations) for extreme small particle applications. Ideal for extracellular vesicle research and measurement of large virus.
- Micro model (A50 or A60 generations) offers good light scatter sensitivity for a wide range of applications including microbiology applications.
- Universal model (A50 or A60 generations) is ideal for conventional applications requiring up to 3 lasers, 2 light scatter and 4 fluorescence channels.
- A40-Micro model has been developed for research scientists looking for high resolution and sensitivity from a single laser. Ideal for bacteria, archaea and other small particles
- A40-Universal model offers up to 3 lasers, 2 LS and 4 FL, ideal for conventional mammalian, plant or yeast cell applications.
- A20-Military model (Cat# 9018) was developed for the detection of microbial pathogens in tough environments with minimal operator training. This self-calibrating system includes an automatic data analysis algorithm.
- Auto40 (Cat#9900) has been developed specially for HIV immunophenotyping and offers automated calibration and data analysis algorithms. For this model please refer to the Auto40 Operating Manual (Apogee Doc 16008, Cat#9915).
- Micro-GxP model for use in regulated environments. Refer to the operator manual dedicated to this model: ApogeeFlow document #16057.

1.3 Regulatory & Safety Information

Quality System

Apogee operates under a Quality Management System based upon BS EN ISO 13485:2003 Medical devices. Apogee flow cytometers are for research use only unless specifically labelled as suitable for other uses.

CE Marking Policy

Unless specifically labelled for other purposes, ApogeeFlow products are CE marked for Research Use Only.

Laser Safety & Classification

Apogee flow cytometers are Class 1 laser products; there is no laser hazard. During operation the user is not exposed to laser light. For maintenance procedures requiring removal of the covers, it is recommended to first turn all lasers off using the check boxes on the Control Tab of Histogram Software. If the covers are removed and laser(s) are turned on, the machine is then a Class 2M laser product; the system is safe for accidental exposure providing optical instruments are not used to view or divert the laser light.

FCC Regulatory Information

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Apogee flow cytometers have been tested against Code of Federal Regulations 47, Part 15, Subpart B 15.107(b) and 15.109(b).

EMC Compliance

Apogee flow cytometers have been tested and found to comply with

EN 61326-1:1997 including Amendment 1
EN 61000-3-2:2000
EN 61000-3-3:1995+ A1:2000

indicating their emissions of and immunity to electromagnetic radiation are within the limits defined by these standards.

Furthermore, this device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Any electrical device is capable of causing electromagnetic interference with other electrical devices and can themselves be affected by other electrical equipment. Care should be taken to minimize such effects when choosing a location for the machine.

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DECLARATION OF CONFORMITY

We declare, under our sole responsibility, that the products identified in this declaration and to which this declaration relates are CE labelled in conformity with the European Council Directives.

Machinery Directive (2006/42/EG)
Electromagnetic Compatibility Directive (EMC) (2014/30/EU)
RoHS (2011/65/EU)

Description of Equipment:

Universal Flow Cytometers
Micro Flow Cytometers
Micro-PLUS Flow Cytometers

This declaration covers the above products manufactured from 1ST January 2019

Standards applied:

IEC EN 61010-1:2010 + A1
Emissions standard: CISPR 11 / FCC Category: Class B
Immunity standard: EN 61326-1:2013 Category: Basic

The product is manufactured in the EU by:

Apogee Flow Systems Ltd.
Unit 7 Grovelands, Boundary Way
Hemel Hempstead, HP2 7TE, UK

Apogee operates under a Quality Management System based on BS EN ISO 13485:2012 Medical Devices.

2nd January 2019

Date of issue

O. Kenyon
CEO

FDA 21 CFR Part 11 Compliance

Apogee's flow cytometer user interface, the "Histogram" application, may be configured to operate in a "GxP" mode which is intended to meet the requirements of part 11 of Title 21 of the Code of Federal Regulations; Electronic Records; Electronic Signatures (21 CFR Part 11). The software is not certified to this standard but when configured in "GxP" mode, the Histogram application generally meets these requirements.

This option is offered as Apogee product # 8057 and requires that the system be specially configured by an Apogee field service engineer.

Disposal of Electronic Waste

This product contains electronic items which must be disposed of in compliance with local regulations. Within the European Union, Apogee is pleased to arrange for the collection, treatment, recycling and environmentally sound disposal of Waste Electrical and Electronic Equipment (WEEE) manufactured by us.

Disposal of Waste Products

Waste fluid from the flow cytometer will contain potentially hazardous biological contaminants. Dispose of carefully and according to local regulations.

Treat used sample tubes and pipette tips as hazardous biological waste according to local regulations.

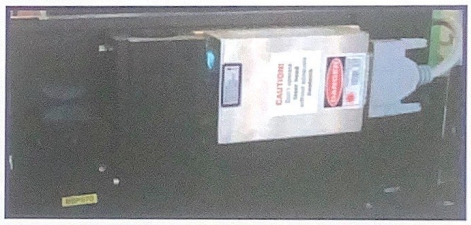
2. Technical Overview: Optics

2.1 Flow Cell

The flow cell is at the heart of the machine and consists of a quartz crystal through which a flow channel allows sheath fluid, with sample fluid & particles at its central core, to flow. The flow cell assembly is designed for maximum stability. If a fault or damage occurs, the "OL&FC Assembly" (Objective Lens and Flow Cell Assembly) can be replaced by a service engineer. The damaged unit should be returned to the factory for re-conditioning.

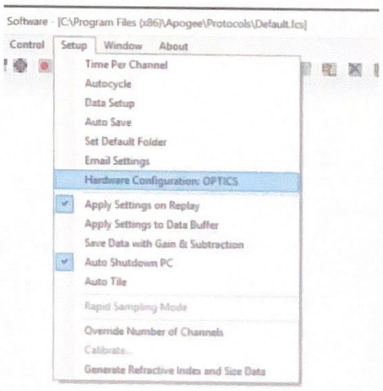
2.2 Lasers

Apogee uses only the latest solid state laser technology. Typical laser lifetimes exceed 10,000 operating hours. Lasers may be mounted on the front and back of the optical bench plate and may have some adjustment mechanisms for service engineer use. Refer to section 1.3 for laser product classification information.



2.3 Optical Configuration & Fluorescence Filters

The precise optical path depends on instrument model and configuration, but a schematic representation is shown here for an A40 generation cytometer with 3 fluorescence detectors. The photomultiplier detectors are mounted on the rear side of the optical bench (not shown).

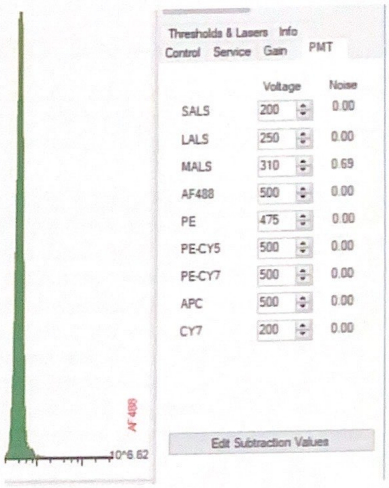


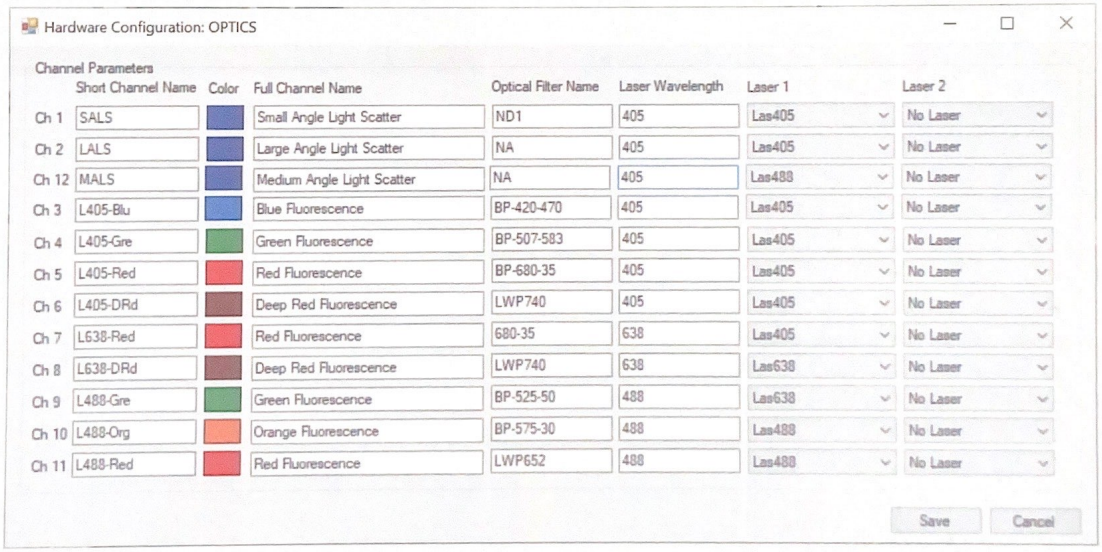
Your cytometer's particular optical configuration can be displayed via the Setup menu and also by double clicking on any of the parameter names on the "Gain", "Service" or "PMT" tabs on the Control Panel of the Histogram Software. The configuration information is stored in the FCS file.

This dialog can be used to enter "Short Channel Names" which reflect the fluorophore you are measuring.

Multiple lasers focused to the same spot (coincident lasers) should be specified as comma separated values in the Laser Wavelength control. Use numbers and commas only in this control, no other characters.

Do not use the "/" character in any of the text because this character is used for formatting in the fcs standard.

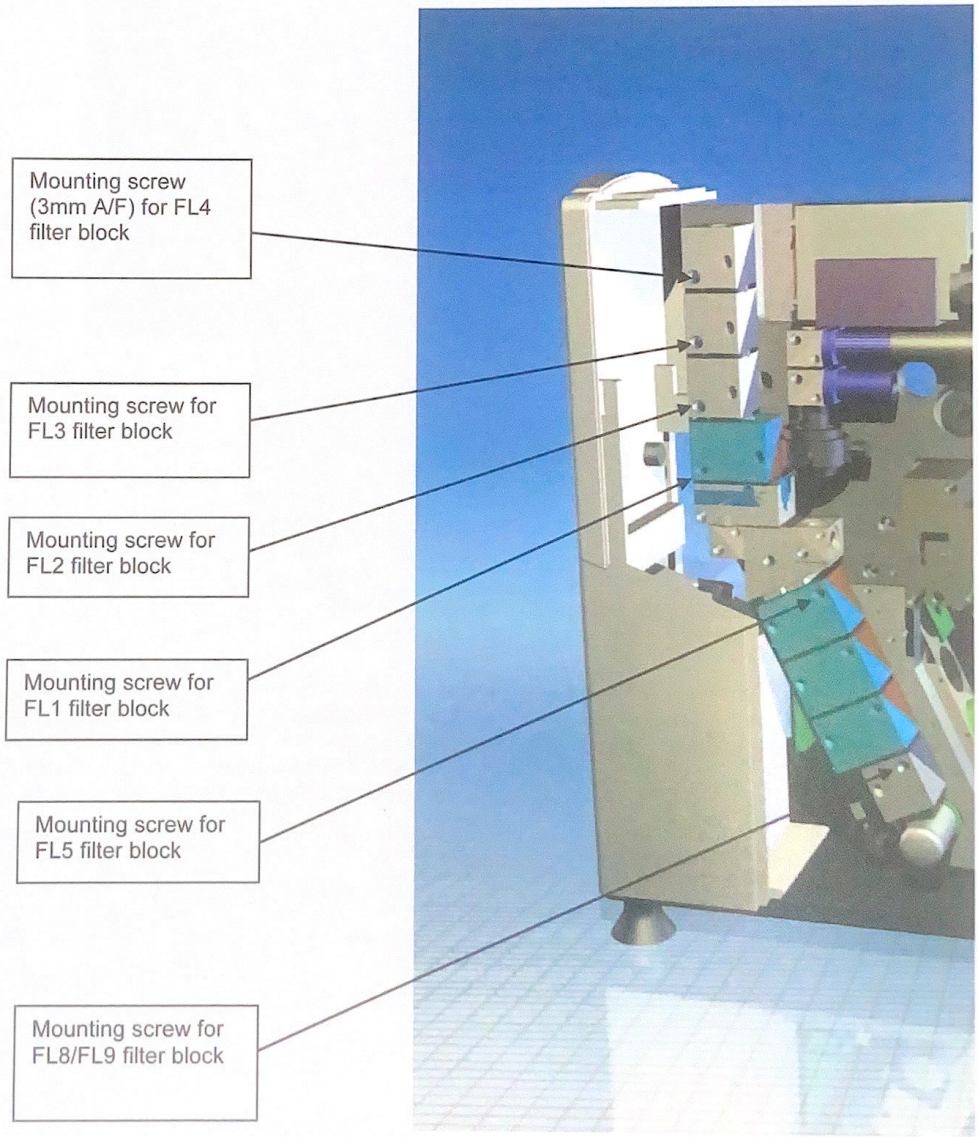




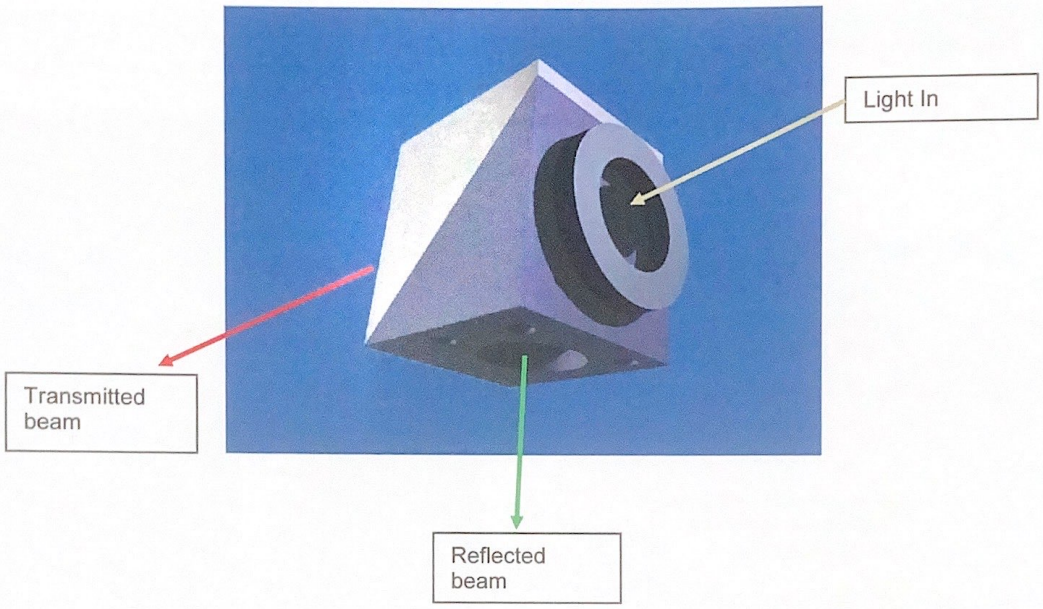
2.4 Fluorescence Filter Blocks

The operator should be aware of the fluorescence filter block specifications which are written on the filter blocks and set in the software via the 'User Configuration' program (shortcut on desktop). Filter blocks can be changed by the operator using the Allen key (3mm A/F) supplied with the instrument, without the need for realignment. The user must then remember to edit the software descriptions via the User Configuration program.

There are two types of filter block: 'Single beam' or 'Dual beam'. In the below picture, there are single beam filter blocks in positions FL2, FL3, FL4 and FL8/FL9.



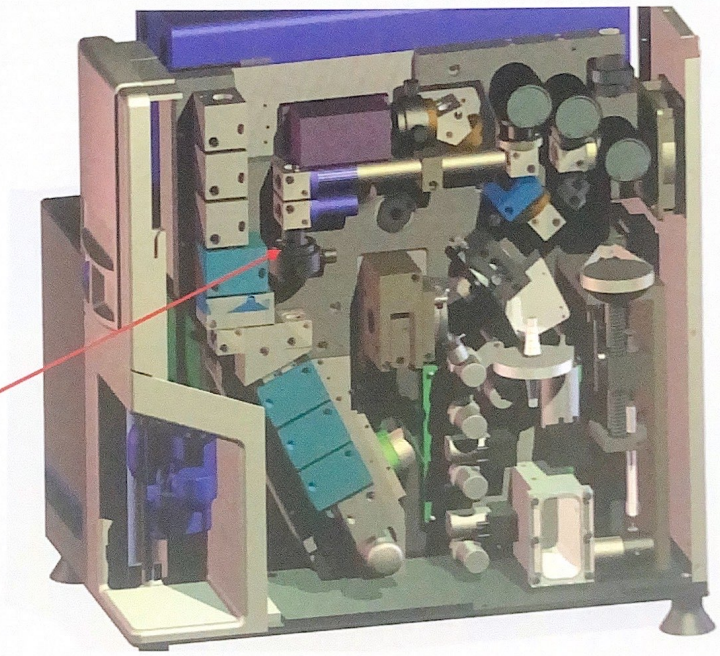
Single beam fluorescence filter block:



2.5 Light Scatter Iris Field Stop Adjustment

The A50 and A60 generation optics are fitted with an iris in the light scatter path which can be adjusted by an advanced user to optimize the system for different applications. Small particle applications benefit from a smaller aperture (better signal to noise ratio) and large particle or high sample flow rate applications benefit from a relatively larger aperture size. The iris size can be adjusted using the lever. It is recommended to only make this adjustment when running calibration beads (e.g. 1um) and viewing the image on the flow cell camera.

Lever to adjust light scatter aperture. View camera image while adjusting. Smaller aperture gives better signal to noise ratio, larger aperture gives better high flow rate data precision (CV).



3. Technical Overview: Fluidics

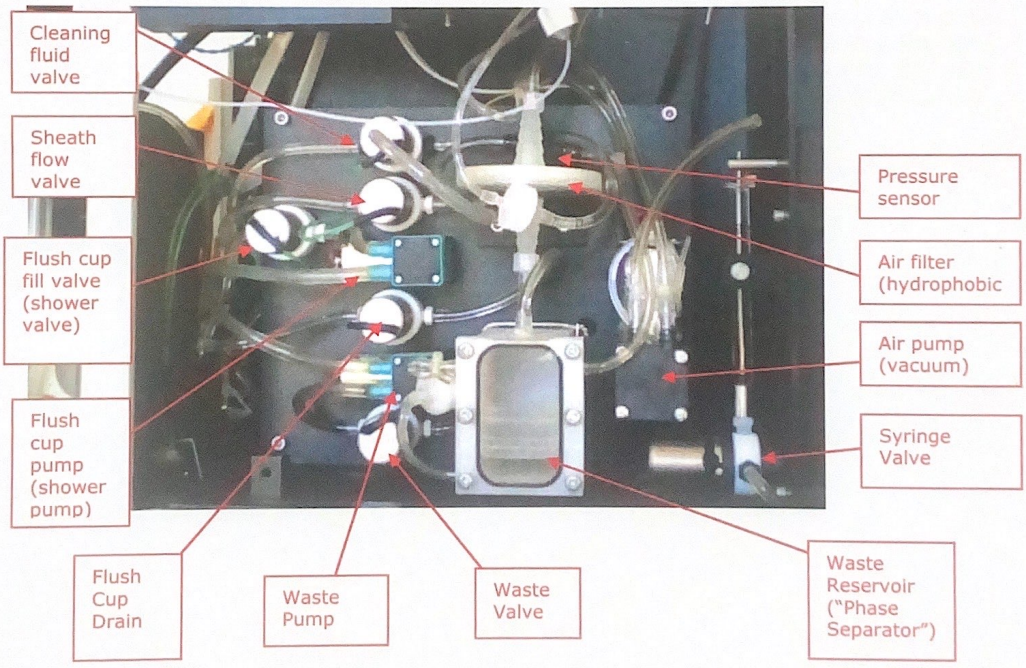
The fluid system is designed for bio-safety, simplicity and reliability. Sheath fluid is driven by a regulated vacuum pump. Sample is delivered to the flow cell by a high precision syringe which allows the software to calculate the absolute number of particles (events per microliter).

3.1 Fluid System

The instrument can be fitted with a choice of fluid systems: A closed loop "Long Life" fluid box or a refillable tank.

The layout of the pumps and valves differs between the A40, A50 and A60 generations of the cytometer. The A60 layout is identical to the A50 layout except that the fluidics in the A60 generation is located at the front left of the cytometer instead of the back right. See images below.

A40 Fluidics



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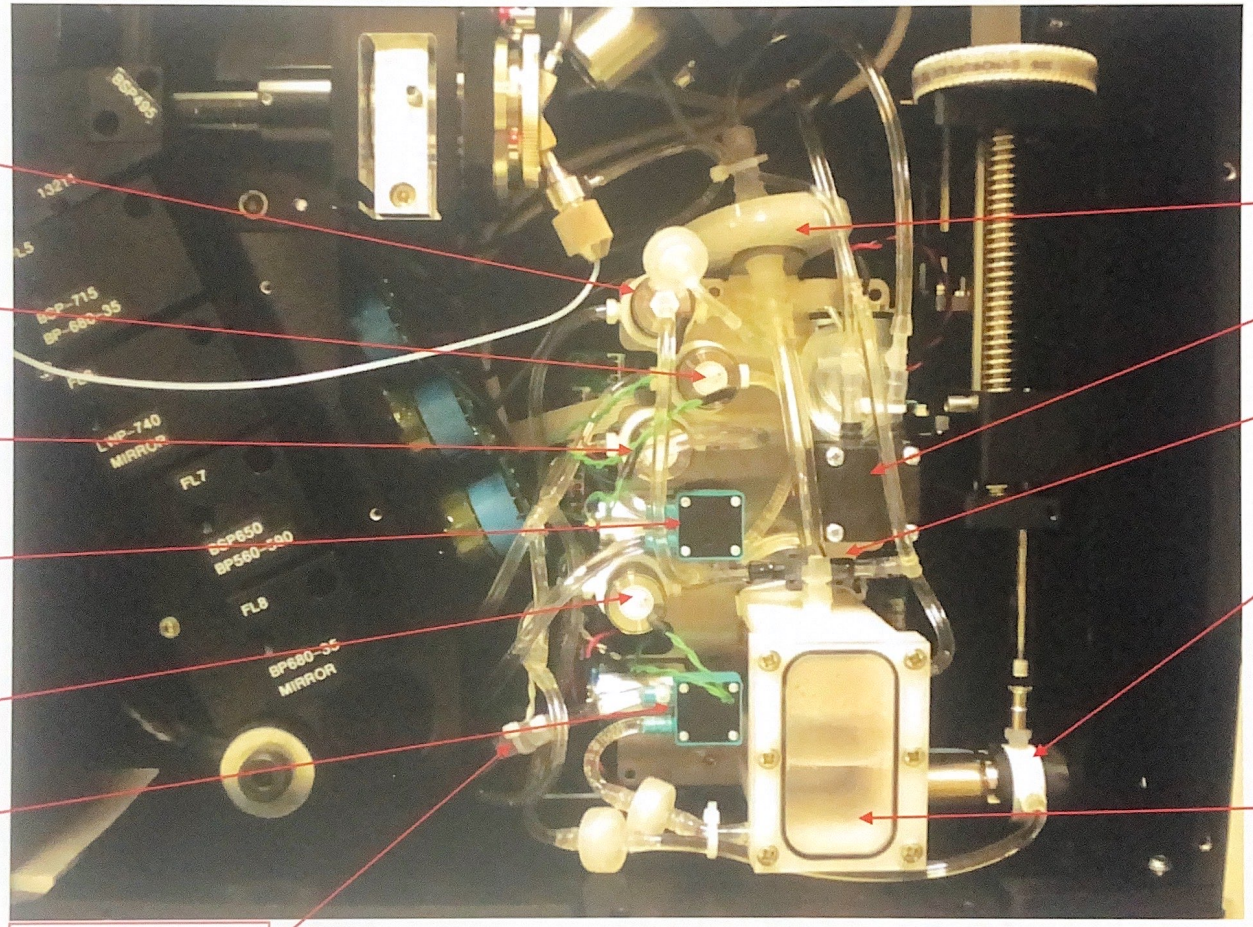
A50/A60 Fluidics



Cleaning fluid valve

Sheath flow valve

Flush cup fill valve

Flush cup pump

Flush Cup Drain Valve

Waste Pump

Waste Valve (1 way)

Air filter (hydrophobic)

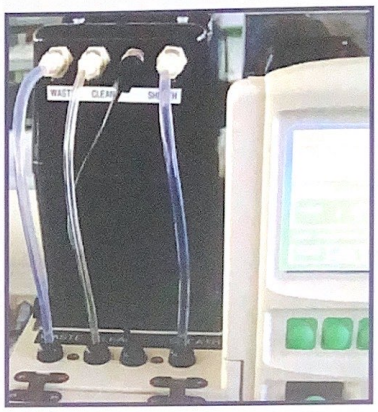
Air pump (vacuum)

Pressure sensor (black)

Syringe Valve

Waste Reservoir ("Phase Separator")

3.1.1 Closed Loop "Long Life" Fluid System



If the instrument is supplied with this type of tank, connect the tubes and the electrical cable following the labels on the instrument and on the connectors.

Always turn the machine's power off before connecting or disconnecting the fluid box / tank.

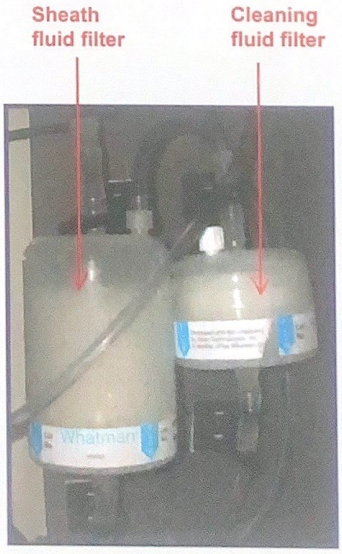
The machine will give an 'Out of Sheath' message on the instrument's display when the tank reaches its end of life.

After changing the tank, turn on the machine and allow it to initialize. Select "New sheath" from the machine's display options before running your samples.

With this system no operator intervention is required until its internal sensor detects the end of life.
At this time the operator is given an "Out of Sheath" message on the instrument display because the fluid box can supply no more sheath fluid. When this happens, shut the machine down, replace the fluid box and dispose of the old fluid box according to local regulations, bearing in mind that its contents are likely to contain hazardous biological and chemical material from the samples run.
After fitting a new fluid box, select 'New Sheath' from the instrument's display to properly prime the system.

The long life fluid box can be ordered as a pack of 4 boxes: Apogee Cat#1422.

3.1.2 Sheath Fluid and Cleaning Fluid Filters

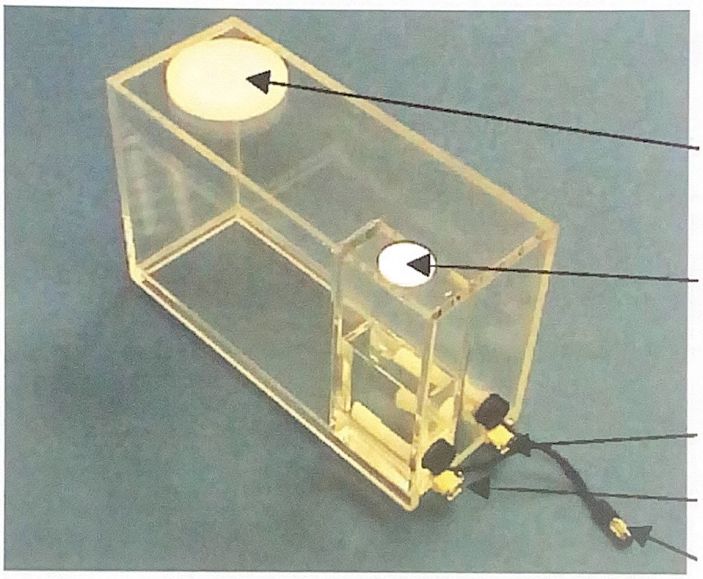


The fluid filters have their inlets connected to the fluid tank by tubes which pass through the side panel. The location of these filters varies between flow cytometer models. The cleaning fluid filter typically lasts more than 6 years. The sheath fluid filter should be changed if there is an elevated amount of sheath fluid dirt measured by the machine when running filtered water, or every 3 years.

The sheath fluid filter of the type shown in the photograph is hydrophobic so it will not allow the flow of water if it fills with air (refer to section 11.5 for test and wetting procedure).

3.1.3 Refillable fluid tank

The refillable fluid tank requires the use of an external waste tank.

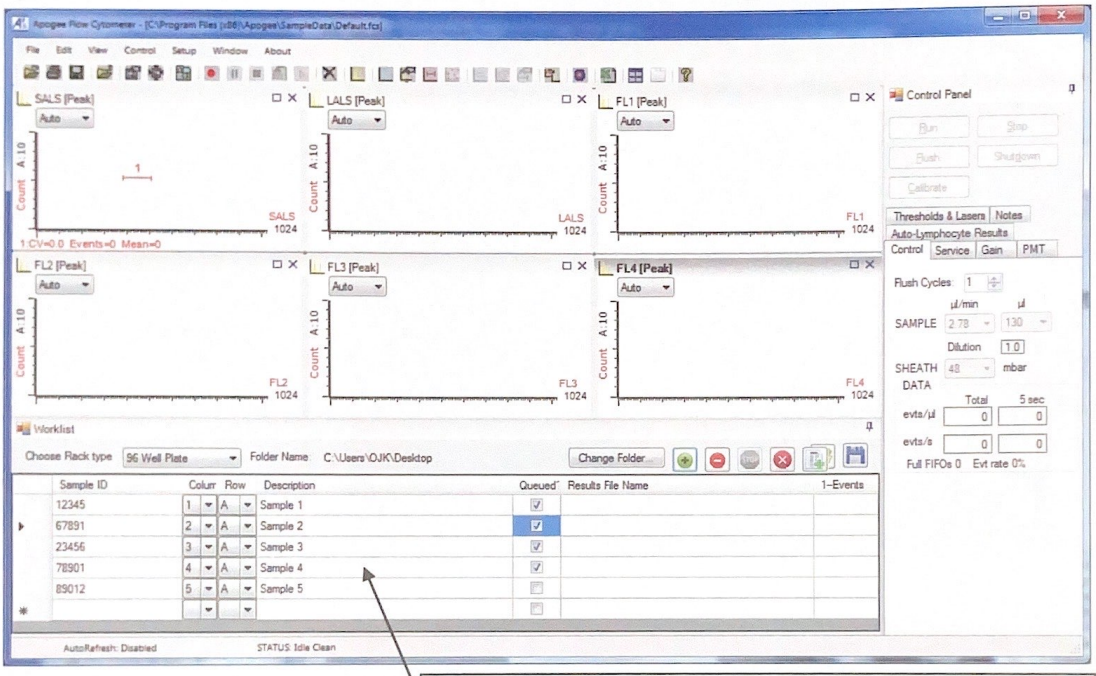


To install the refillable tank:

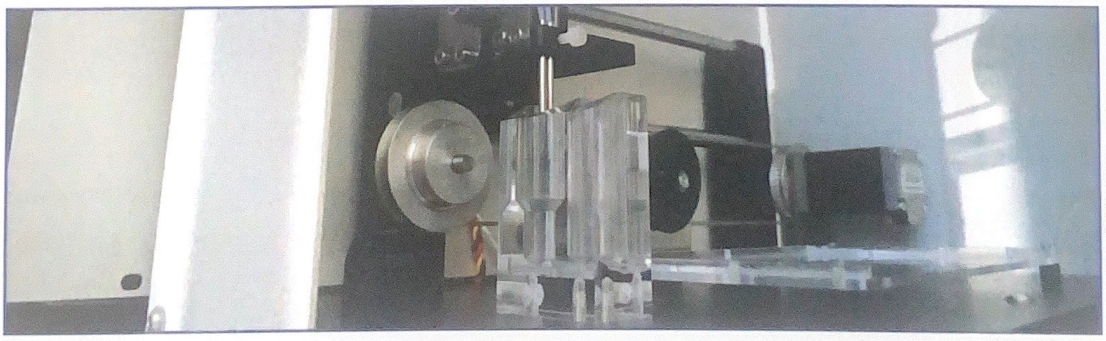
- Fill the sheath fluid reservoir with deionized, filtered water or with other Apogee approved sheath fluid. Add 200µl of Proclin 300 (Supelco) as a biocide to discourage growth in the water. Use treble the normal dose of biocide if growth is suspected or if growth has been detected within the past 2 weeks.
- Fill the cleaning solution reservoir with Apogee cleaning fluid (Cat# 1519) diluted to the concentration on the label (typically 30%)
- Connect the cleaning solution reservoir and the sheath fluid reservoir to the appropriate machine hose connectors.
- Connect the level detection cable to the machine's cable
- Check the waste tank is empty and connected.
- Turn on the machine and allow it to initialize.

3.2 Autosampler Module for 96 well plates (Optional)

This optional module allows automatic sampling from 96 well plate format wells or racks of tubes. It requires software with Work List functionality. The hardware has sensors to make sure the cover is closed before it operates and sensors to detect failures of the sample aspiration tube movement mechanism due to a poorly positioned sample rack or some other fault condition. Refer to the "Autosampler Operation" chapter of this manual for further information.



Work List
Samples must be given an ID, a location and must be Queued in order for them to be processed.



4. Technical Overview: Data Acquisition

The height and area (integral) of pulses from the photomultiplier tubes are measured using high performance analog-to-digital converters (ADCs) giving 18-bit pulse height resolution and 26-bit pulse integral (area) resolution. This is sufficient data resolution to allow gain factors (linear and logarithmic) to be performed in software thus avoiding the errors that would be introduced by electronic gain circuits.

4.1 Gain Settings

Fine adjustments to gain and subtraction settings do not have to be made until after the sample has been run, and the data stored - these settings can be applied at a later time, without the machine connected.

Of course, if you prefer, gain and subtraction settings can be adjusted during the sample run, but it is often useful to be able to make adjustments at a later time, and the software makes this easy.

Data can be stored with or without gain and subtraction settings applied to the data.

Linear software gain factors are a useful complement to the histogram 'Zoom' function (available by right clicking on any datagram).

4.2 PMT Voltages

The PMT Voltages determine the gain of the photomultiplier tubes: the number of electrons release per photo of energy reaching the photocathode. If the Voltages are set too low the signal to noise ratio will be worse. If the voltage is set too high the PMT tube output may saturate. To prevent saturation of the PMT tubes, keep the PMT Voltages low enough for the PMT Noise levels to always be less than 1.00.

4.3 Event Rates and Severe Coincidence

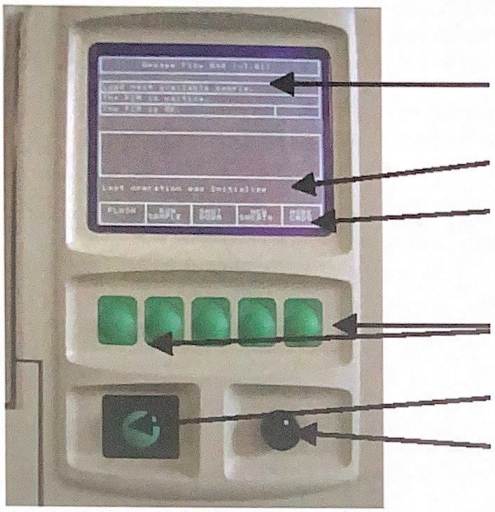
There are three reasons why the cytometer counts:

1) A particle passes through the laser and gives a signal greater than the trigger threshold
2) The trigger threshold is close to system noise and a tiny noise fluctuation has caused the voltage to pulse momentarily above the trigger threshold
3) The sample is causing severe coincidence (AKA 'Swarm' effect) where there is a stream of concentrated particles in the laser; multiple particles in the laser at the same time. In this case a dilution of the sample is necessary or a dramatic reduction of the sample flow rate.

If the source of the particles is (1) then the event rate should be roughly proportional to the sample flow rate (assuming the event rate is well below the electronic limit).

If the source of the particles is (2) then the trigger threshold should be raised to eliminate the noise or keep it to a low level. This type of event will continue to occur when running a sample of water. If the noise has increased (a deterioration of the optical condition of the machine) then you should see that as a change in the PMT noise levels (PMT tab of histogram software). To make sure the noise values are correct, change any of the PMT Voltages by, for example, 1 Volt and wait a few seconds for the noise readings to update. This forces the system to read the noise levels, otherwise the noise levels may not be current.
Sometimes the noise readings can be misread but if you repeat the process of changing a Voltage by 1 Volt several times, the true value will be apparent. A small change in the noise level will cause a massive change in the event rate if the trigger threshold is close to noise.
It is therefore possible to establish (by changing the sample flow rate or running water and by monitoring the PMT noise readings) the source of events: genuine particles, optical noise (either background light level or severe coincidence effect).

5. Display Panel (A20 Military model only)



An instrument display can be installed to display basic instrument status information. If specially programmed, it can also be used to display information about populations of particles in the samples ('stand-alone' mode).

Under the display there are 5 green buttons. These are not normally needed because all functions can be selected from the histogram software.

Most models do not have this type of display because all settings and data are accessed via the histogram software on a separate LCD monitor.

5.1 Commands available from the Display Panel

FLUSH
Flushes (cleans) the sample tubing. Ensure the sample arm is in the flush position.

RUN
Runs a sample. Ensure the sample arm is in the run position and sample is available in the test tube. It is not recommended to allow the system to suck up air instead of sample.

SHUTDOWN - Normal / Cold Shutdown
Selecting 'Shutdown' and then 'Normal' performs a Flush, delivers cleaning fluid to the flow cell and prepares the machine for turning the power off.

'Cold Shutdown' is for storage of the machine in temperatures below 5 degrees Centigrade. A special fluid box (containing antifreeze) is required for this operation.

NEW SHEATH
Select this command each time you change the fluid box (long-life, 'closed loop' fluid box). It runs a various fluidics cycles to remove air from the sheath fluid system, and to flush through the sheath fluid tubing.

MORE CMDS
Displays additional commands, e.g. 'Flow Cell Clean'

FLOW CLEAN
Delivers cleaning fluid to the flow cell, lets the flow cell soak in cleaning fluid for approx 40 seconds and then flushes the sample tubes and flushes the cleaning fluid through the flow cell.

INIT
This command performs various cycles to prepare the machine for the first sample of the day. If the machine is configured to run in Stand-Alone mode then a Calibration (CAL) is automatically performed at the end of this Initialization routine.

CAL
Pressing "MORE CMDS" followed by "CAL" toggles Calibration mode on/off.
The machine is in normal sampling mode when "RESULTS:" is displayed on the LCD, and Calibration mode when "CALIBRATE:" is displayed on the LCD.

6. Cytometer Setup

Choose a safe location for the machine with at least 20cm of ventilation space behind the machine. The instrument's two fans (back panel) require 20 cm of ventilation space; otherwise the machine's processor may overheat causing the instrument to shutdown without fluidics cleaning cycles.

6.1 Unpacking

The system should be unpacked and installed by an ApogeeFlow trained employee or local representative. See section 11.6 for repacking instructions in case the system needs to be transported.

6.2 Instrument Back Panel Connections

The instrument can be run either:

1. Connected to an external PC.
2. Using the internal PC for data analysis with an external monitor, keyboard and mouse connected to the back of the machine.

A PC, running *Windows* is built into the machine, so no external computer is required. However, an external PC may be configured if LAN security demands.

Set-up for Histogram Data Analysis Using Internal PC

1. With the power off, connect a monitor, mouse and keyboard to the connectors on the back of the machine.
2. Turn the power on and wait for the operating system and FCM Control software to boot up.
3. Allow the machine to initialize.
4. After the initialize cycle has finished, the machine is ready for a sample. Run calibration beads to check instrument operation.

Using an External PC for Data Analysis and Storage

Use of an external PC is possible if LAN security demands.

Communication between the external PC and the machine is via a standard LAN connection. To connect the external PC to a LAN, a second network card is required.

The network name of the cytometer's PC is set in the factory to 'Axxxx' where xxxx are the first four digits of the instrument serial number.

The instrument's IP address settings will normally be sent with the machine. Alternatively they can be found by connecting a monitor, keyboard and mouse and using the Windows operating system (Control Panel—Network Connections etc.).

6.3 Network Configuration

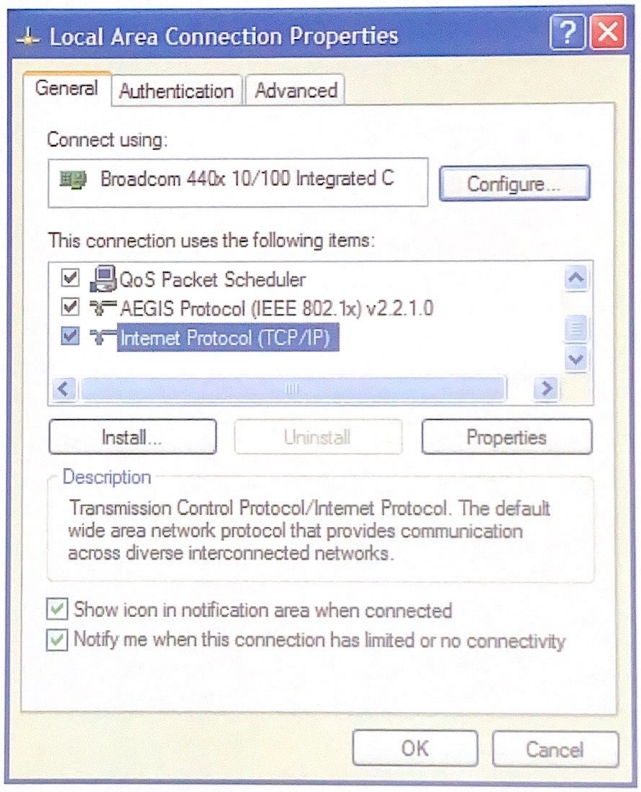
A network connection may be established with the cytometer to take advantage of the following features:

- Use of remote desktop software to administer, service and diagnose the cytometer
- Data collection and instrument control using the Histogram Software running on an external PC
- Transfer of datafiles AutoSaved to the cytometer's internal disc drive

The cytometer can be connected to a local area network (LAN). It can be configured with a fixed IP address or the IP address can be DHCP assigned. This is configured using the Windows operating system.

Important notes:

- All network connections must be established BEFORE the cytometer's 'FCM Control' software starts. So connect the network before turning on the machine.
- If the PC is configured to connect to a network with special security settings, consult the network administrator. Some network security systems may block communications between the FCM Control program and Histogram Software.

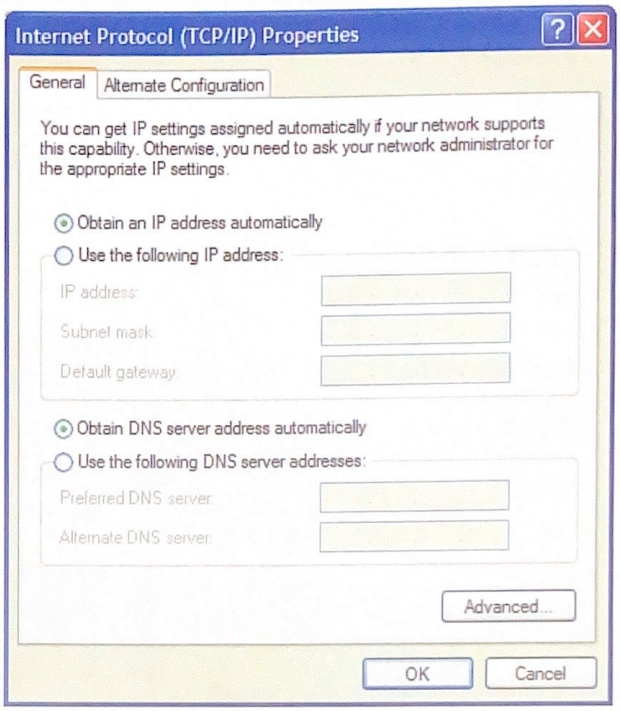


Appropriate network settings will usually be made in the factory. To check or change the network settings, go to the properties of the network connection:

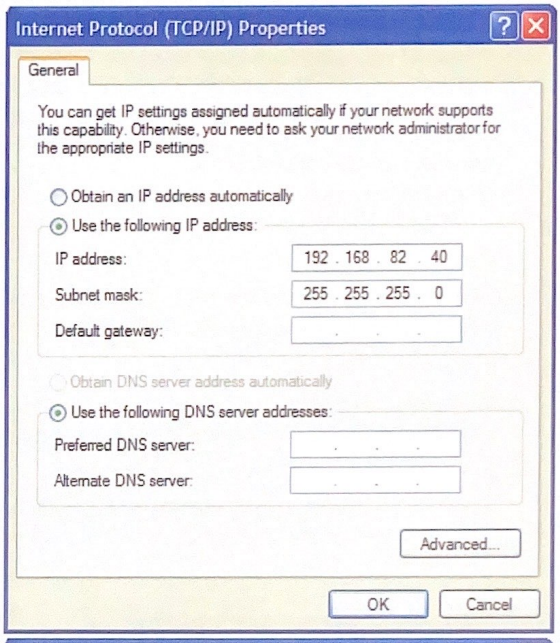
Scroll down the list of items, choose Internet Protocol (TCP/IP), and click Properties.

If the machine is being used on a LAN with a DHCP server the cytometer will obtain an IP address automatically.

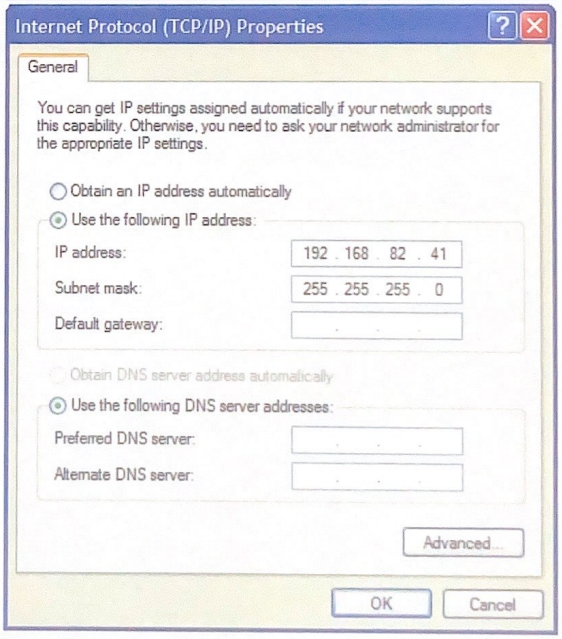
If the machine is being used on a LAN without a DHCP server (for example if the machine is connected directly to another PC with a 'twisted' network cable, or via a network switch or hub) a fixed IP address must be used.



When using a fixed IP address for the cytometer, 192.168.82.40 is often used.

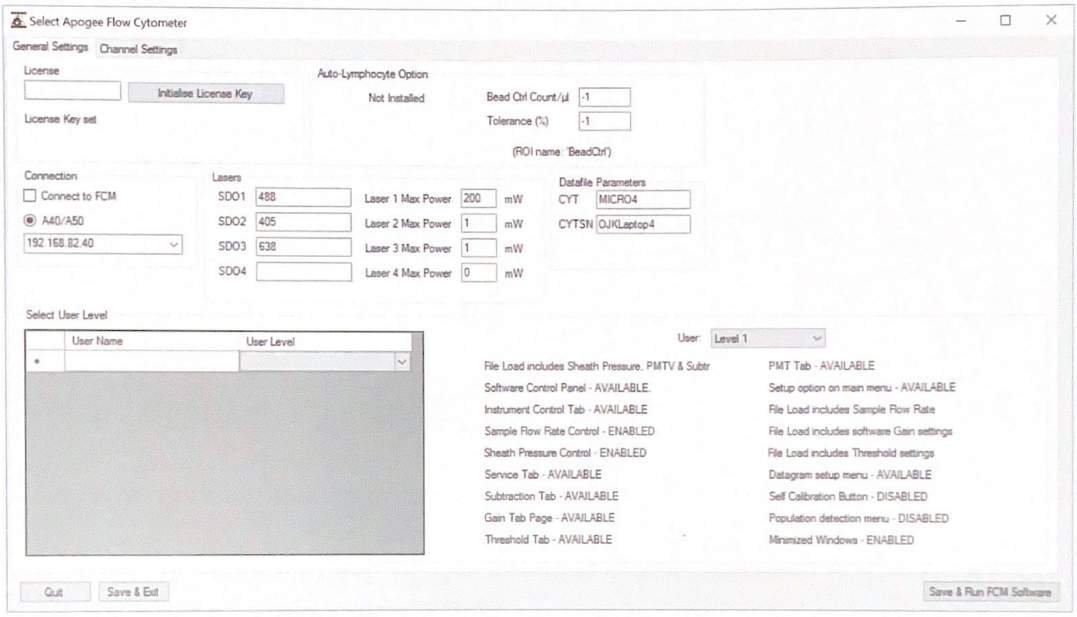


The IP address of the PC which you intend to use to connect to the machine, must be set to a similar address, but different. For example 192.168.82.41 as shown below:



If fixed IP addresses are used as above, without a DNS server specified, it may not be possible to use the network name of the machine to access it. Instead the machine's IP address should be used.

In the histogram software's User Configuration program, the IP address can be entered instead of a network name as shown below.



7. Apogee Software

The machine's hardware is controlled by a program called 'FCM Control' which runs on the computer inside the cytometer. It runs in the background and requires no user interaction.

Software for collecting the data in industry standard FCS format and for viewing the data on histograms can either be run on a separate PC connected by Ethernet link, or on the internal PC. This histogram software comes with a User Configuration program.

The **FCM Control** program requires permissions and drivers to talk to the electronics and transmit data to the Histogram application. The FCM Control program is factory configured and no operator interaction is required.

The **Histogram** application talks with the FCM Control program via TCP/IP. The address or network name of the cytometer PC must be entered in the User Configuration program to allow the Histogram app to connect to the FCM Control program.

The software is installed in the factory so does not need installing by the operator but section 7.2 below explains how the Histogram app may be installed on separate PCs for convenient data analysis, avoiding the need to use the cytometer PC.

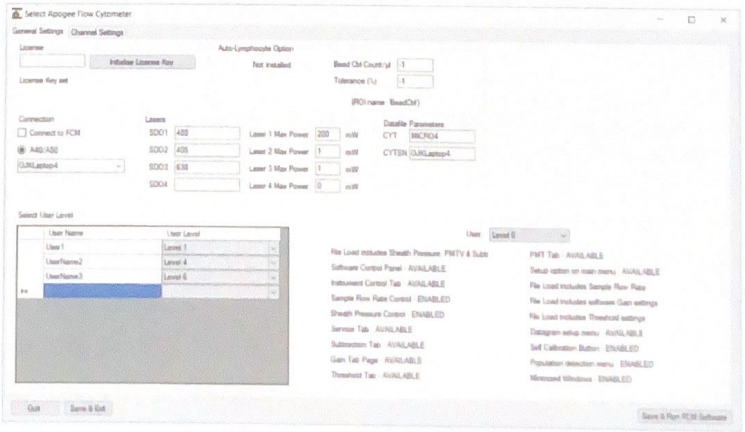
7.1 Multiple Windows User Accounts and GxP Mode

7.1.1 Multiple Windows User Accounts & Permissions

To configure a new Windows User, set the new user as an Administrator to start with. Run the programs "HistogramInitialiser.exe" and "HistogramUserLicense.exe" when logged on as the new user (with Admin permissions). Make sure the Histogram application runs and set the default file folder when prompted when the application runs for the first time. At this time it is helpful to set the Autosave folder via the WorkList header button.

Only once the Histogram application has been run as a genuine Admin user can the user's permissions be reduced to a Standard User. See section 7.2 below.

To control the permissions of each Windows user, each user's Windows name should be entered into the User Configuration program and a User Level chosen. Access may then be restricted to the User Configuration program (for example by deleting the program shortcut and/or the executable file) so that unauthorised users are unable to change the User Level. The list of permissions and the User combo box control allow you to see the permissions for each user level.



Windows 10 requires the FCM Control application to be run with Admin permissions for it to communicate with the Autosampler electronics. To talk with the FCM Control application the Histogram application may also need administrator permissions. If so, for Standard Windows users, the Histogram App can be run with Administrator permissions by creating a shortcut with the following path:

runas /user:A0999\ApogeeFlow /savecred "C:\Program Files (x86)\Apogee\Histogram.exe"

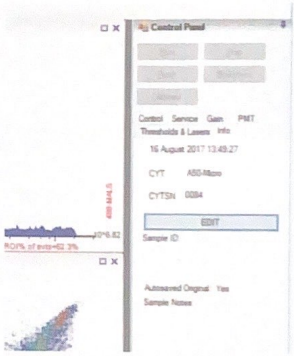
where:

- A0999 is the computer name
- ApogeeFlow is an administrator user

The first time this shortcut is used the user is asked for the administrator password and a Windows credential is created (refer to Windows Credential Manager).

The Histogram application will then run with the user level assigned to the administrator (in this case ApogeeFlow). It is therefore normal to create an Admin account for use by the Standard users and with Level 3 permissions set in the User Configuration program.

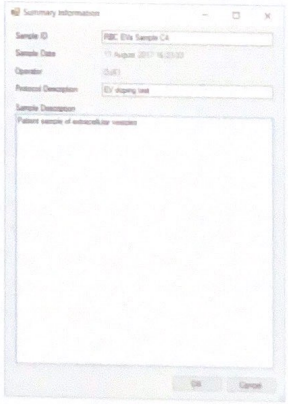
7.1.2 Data Integrity: $ORIGINALITY, $OP, Acquisition Date & Time



The Windows user name is saved in the FCS file with the $OP keyword. In this way the original operator is identified in the FCS file. The user can be found by opening the Summary Information dialog from the EDIT button on the Info tab of the Histogram Software.

When a datafile is saved using the WorkList Autosave feature, the $ORIGINALITY keyword is set true to indicate the file has not been tampered with since the data was acquired. If a file is saved (or re-saved) in another way, the $ORIGINALITY keyword is set to false. The exception to this rule is when "User Level 3 – 21CFR Part 11" is active (see section 7.1.3). When a file with true $ORIGINALITY is opened, the words "AutoSaved Original" are displayed below the SampleID on the Info tab.

The date, time of the data acquisition and Windows user name are also saved into the FCS file and displayed on the 'Info' tab of the Control Panel and in the Summary Information dialog.

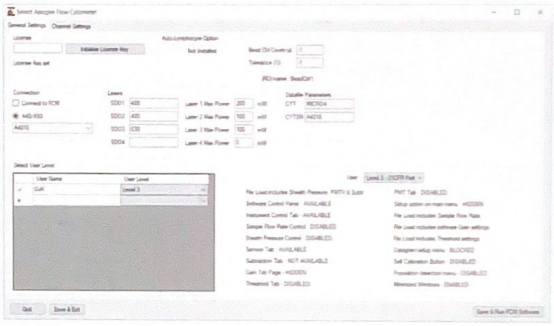


7.1.3 GxP Mode (User Level 3) for Regulated Environments

The Histogram application may be operated in a mode essentially compliant with part 11 of Title 21 of the Code of Federal Regulations; Electronic Records; Electronic Signatures (21 CFR Part 11). This option is offered as ApogeeFlow product # 8057. The Histogram application GxP module requires the system be configured by a service engineer.

Operation in GxP mode requires Windows Users to be set up with appropriate Windows security permissions and each user's operating permissions must also be set using the User Configuration program (see section 7.1.1). Instrument operators should be set to work under User Level 3. Laboratory administrators may be configured with User Level 1.

The User Name column is for Windows User Names. With User Level 1 the user has full permissions.
The 'normal' / routine operator User Level should be set to Level 3 which is for the GxP mode of operation (limited permissions).
After creation of a new Windows user account, when logged on as that new user, the Initialiser app and the User License app must be run to allow the Histogram app for execute. These apps will be provided to the administrator.



When Histogram app is running under User Level 3, the Calibrate button and calibration status indicators are displayed on the Control Panel. To run a calibration you can use the Calibrate button or type a Sample ID beginning with the characters "ApoCAL", then press F10 or click the Run Button. When the ApoCAL sample runs with the machine in Calibrate mode, the software algorithm looks at the shape of populations in two dimensional regions and assesses the number of particles in any populations identified in each region. If the results from each region meet requirements defined in the FCM Control program, the system's calibration status is set to "CALIBRATED" at the end of the sample run.

Detailed calibration results are shown in the FCM Control Window as the sample runs and are saved in a log file to allow long term monitoring:

C:\Users\Public\Documents\Apogee\CalibrationLog.txt

For service engineer use, technical data from every failed calibration is stored in file:

C:\Users\Public\Documents\Apogee PC Control\logError.txt

While running a calibration sample (during the calibration sample data acquisition), when the software detects a population in one of the internally defined regions it compares the brightness of the signals with internally defined target locations and automatically adjusts the photomultiplier voltages if the population is not in the correct position. After each such calibration of the signals, the FCM Control program sends a command to the Histogram application to clear the screen of data and continue analyzing the calibration sample with the new photomultiplier voltage settings. The small adjustments made to the PMT voltages 'calibrate' the signals so they are the same strength each day. If the software deems a large voltage change to be necessary the calibration will fail.

The calibration status changes to GREEN if the calibration is successful or RED if it fails. The calibration status is stored in the fcs datafile. The calibration will be set to expire after a defined interval, typically 8 hours.

Once calibrated, the user may run their samples, but they cannot generally change instrument settings; they must load a predefined settings Protocol file from the File--->Settings menu option or by selecting a protocol in the WorkList.

Administrators should be aware that:

- The list of protocols is a list of the fcs files in folder "C:\Program Files (x86)\Apogee\Protocols". An administrator (Level 1) user must define those fcs protocol files for use by all users.
- The User Configuration application should be deleted from non-Administrator users' desktop and Start menus.
- A local network administrator should limit write permission to avoid Standard Windows users editing files in folder:

C:\Program Files (x86)\Protocols

The typical operation process is provided in section 8.1.2 and summarized here:

1. Turn on machine and log in to Windows.
2. While waiting for the machine to "Initialise" (approx. 5 mins), click the AutoSave path button in the WorkList header and create a new folder for this session's data.
3. In the WorkList, enter the SampleID "ApoCAL" for the ApoCAL test bead sample (or load a pre-saved WorkList)
4. When the machine is ready for a sample (green indicator on Control Panel), press F10 or click the Run button and wait for the system to calibrate (approx. 2 mins). Make sure the calibration is successful. The data will save automatically to the AutoSave folder when the sample stops.
5. Load a template suitable for the biological samples to be analyzed.
6. Enter SampleIDs in the Work List, these IDs will be used for the fcs file names
7. Run the biological samples. Each sample will automatically save when it completes.
8. At the end of the session, run an ApoCAL sample to verify the condition of the machine
9. Run a sample of 10% bleach to clean the sample tubes.
10. Run samples of pure water until the event rate is low (typically <250/sec) indicating the machine's sample and sheath tubes are clean.
11. Click Shutdown. The machine will perform cleaning cycles and turn itself off after about 5 mins.

This mode of operation is recommended for use of the flow cytometer in a GMP or GLP environment.

In this mode of operation user permissions are limited and an encrypted Audit Trail file is maintained:
C:\Users\Public\Documents\Apogee\Audit.bin
The name of the settings file and all changes and actions made since the last loading of a settings template are recorded in the Audit Trail. The Audit Trail allows users to make certain changes to the datafile (e.g. ROI position changes) without invalidating the $ORIGINALITY keyword.

See section 8.1.2 for information on the ApoCAL automatic calibration.

7.2 Installation of Histogram Software on other PCs (not the cytometer)

Subject to purchase of a valid licence, the Histogram application can be installed on other Windows PCs for convenient data analysis. You may install Apogee's software on computers (other than the computer inside the flow cytometer) as follows:

Subject to purchase of a valid licence, the Histogram application can be installed on other Windows PCs for convenient data analysis as follows:

1. Go to Apogee's software download website (contact Apogee for details). The latest histogram software installation program will be named "Histogram Setup 201xxxxx v255.0.0.xxx.exe". You will also need files "HistogramUserLicense.exe" and "HistogramInitialiser.exe". You will also need a license string specific to your institution.

2. Make sure you are logged on to the computer with full Administrator permissions (it is not sufficient to run the UserLicense.exe program 'As Admin', the user must (temporarily) be set to an Administrator for this license to install properly.

3. Close any open Apogee software and run "Histogram Setup 2015xxxx v255.0.0.xxx.exe" with Administrator permissions to install the main application.

4. Run "HistogramUserLicense.exe" and "HistogramInitialiser.exe" (with Admin permissions). Note that the default location for Windows 7/8/10 (64 bit) is: C:\Program Files (x86)\Apogee and the default for Windows XP and other 32 bit versions of Windows is: C:\Program Files\Apogee

5. Enter the computer license key (only need to do once; it is common to all users) by typing a license key into the User Configuration program. A license key will be supplied by Apogee.

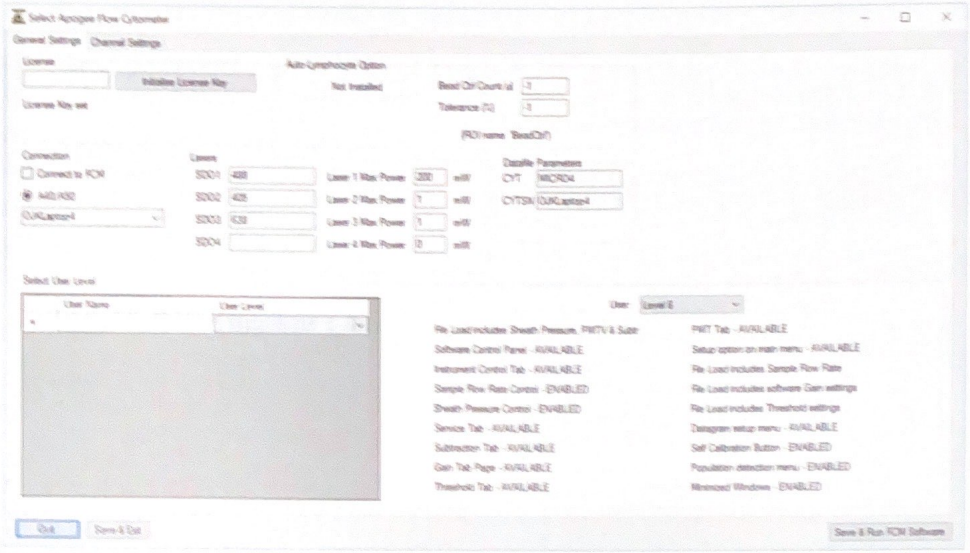
6. Run the User Configuration program and uncheck the "Connect to FCM" box if you do not intend to control a cytometer.

7. The first time you run the Histogram Software, run with Administrator permissions to allow you to set the default file location. You will be asked for your preferred location.

8. Now you can run restrict the Windows User permissions to a lower level.....not before!

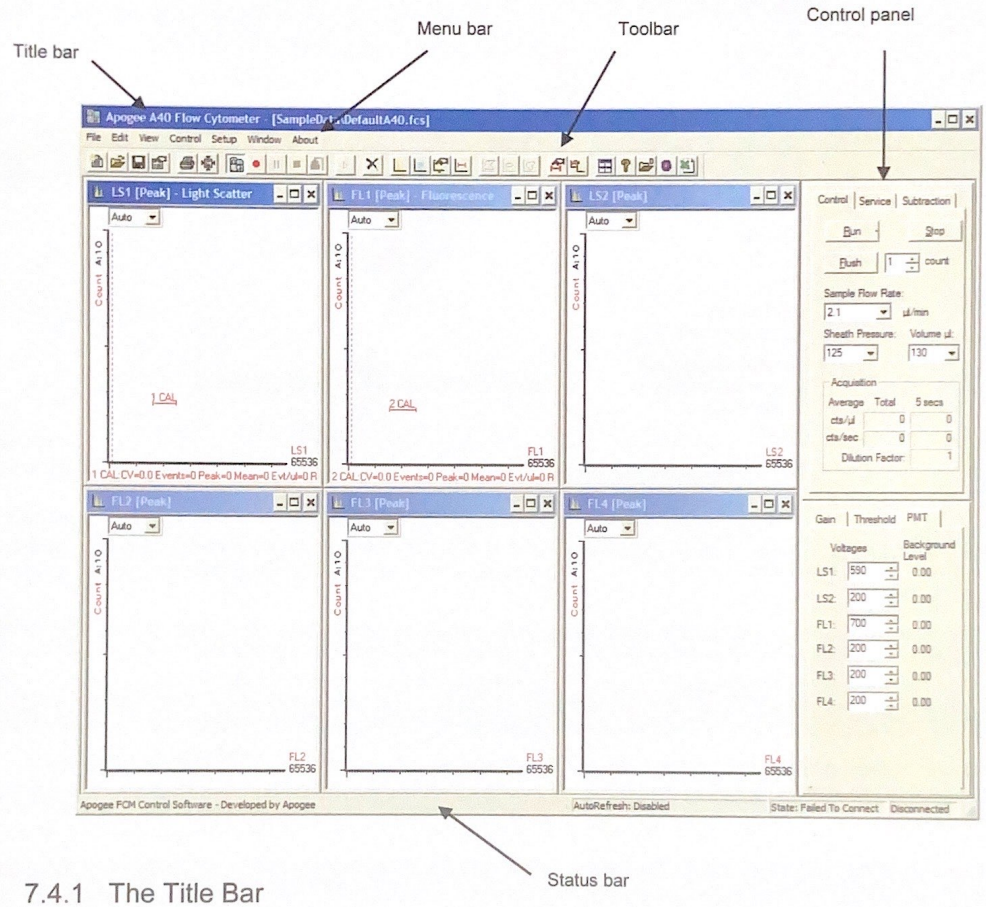
7.3 User Configuration Program

Communication settings for the histogram software are made using this User Configuration program, where the IP address (or network name) of the cytometer is required, and user levels can be chosen. User level 1 is recommended for normal research operation. User level 3 is dedicated to models configured with the GxP option for regulated environments.



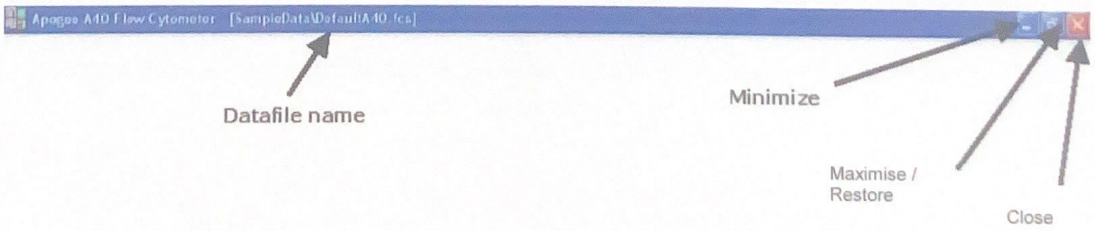
7.4 Histogram Software

The histogram software displays the data from the samples, and allows all the instrument settings to be adjusted. This software is used to read and create data files containing 'list mode' data and instrument settings. Files are stored in the industry standard FCS format.



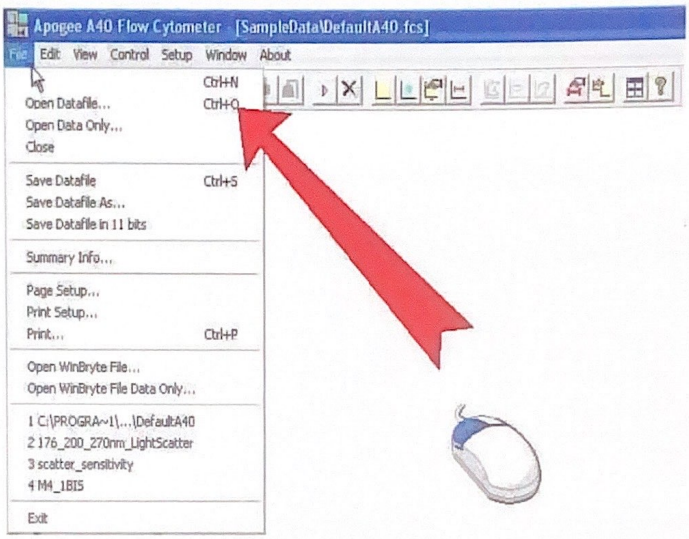
7.4.1 The Title Bar

The name of the last saved/loaded data file is displayed in the title bar. In addition, on the right part of the bar, there are three buttons to minimize, maximize/restore and close the software window.



7.4.2 The Menu Bar

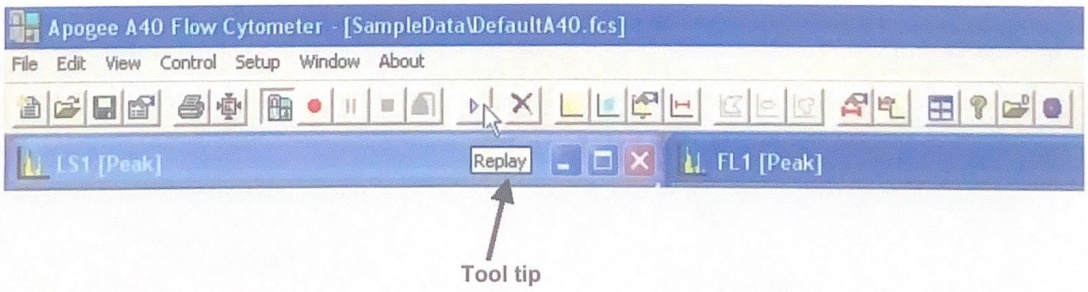
The menu bar contains all the main 'drop-down' menu options.



7.4.3 The Toolbar

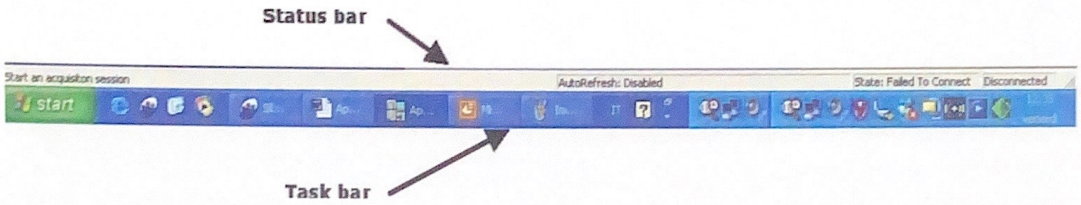
On the toolbar, the most frequently used commands are easily accessible. Just click on one icon to select the related command.

When the mouse pointer rests above one of the icons, a tool tip will appear, indicating the command connected to the icon. In addition, it is possible to see the same indication on the status bar at the bottom of the screen.



7.4.4 The Status Bar

The status bar informs the user about the current status of the instrument. For example: "Running", "Flushing...", "Move arm to flush position", "Shutting Down", "Loading new sheath". If the auto-calibration software is installed, the instrument calibration status is displayed here.



7.4.4.1 Description of Software States

IDLE
There is normally a slow (gravity) sheath flow to keep the fluid system clean.

Flushing
Cleaning sample tubes using the syringe to push sheath fluid through them, also cleaning outside of the sample aspiration tube by filling flush cup with sheath fluid.

Shutting Down
Flushing sample tubes with sheath fluid and cleaning fluid. Cleaning the flow cell with cleaning fluid.

Fluidics Fault
Fluidics cannot reach the normal pressure across the flow cell, e.g. due to blocked sheath fluid flow, faulty vacuum pump or pressure sensor.

Calibrating
The machine is self-calibrating. The calibration will typically take 100 seconds

Calibrated
The machine has passed the calibration test. If the user changes the PMT voltages it will void the calibration.

Uncalibrated
Either has not been calibrated, or user has manually changed the PMT voltages.

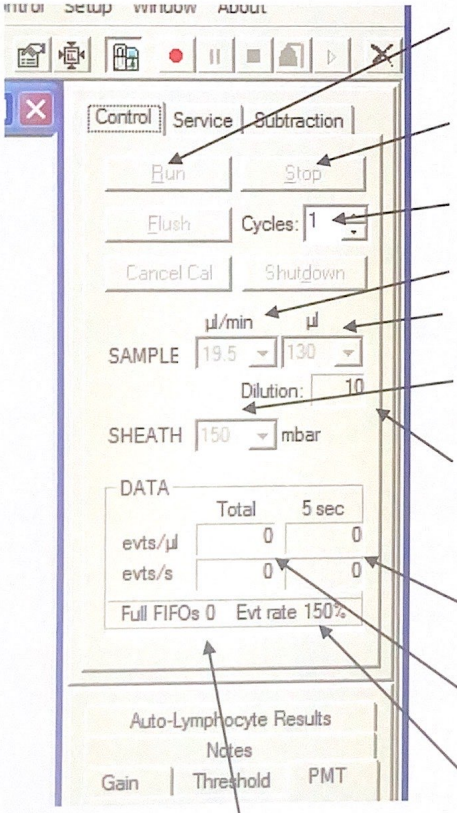
Cal-failed
The machine failed to meet the calibration requirements. Refer to the Troubleshooting section.

7.4.5 The Control Panel

The main instrument settings are accessible from the control panel. If the Control Panel is not visible, click on the View menu and select the option Control panel. The Control Panel is designed to make the most commonly used settings easily accessible. On this panel several tabs are present.

7.4.5.1 Control Tab

This tab is the most frequently used tab.



Run a sample (same as green button display option). Not normally used because samples normally run automatically when the sample arm is moved to the run position.

Stop the sample and flush (same as green button display option). Make sure sample arm is in flush position.

Set the number of sample tubing flushes performed when a flush command is given. If > 4, a rinse operation is also performed.

Set the sample analysis rate

Set the volume of sample sucked up. Note that approx 60ul of sample is not used, so with volume set to 130ul, approx 70ul will be analysed.

Set the sheath flow rate (speed at which cells travel through the flow cell).

Counts per micro-litre dilution correction factor. This is useful if your sample has been diluted by reagent or buffer, and you want to automatically correct the cell concentration displayed by the software. Set this on the Page Setup menu.

Average events per micro-litre and events per second for the last 5 seconds

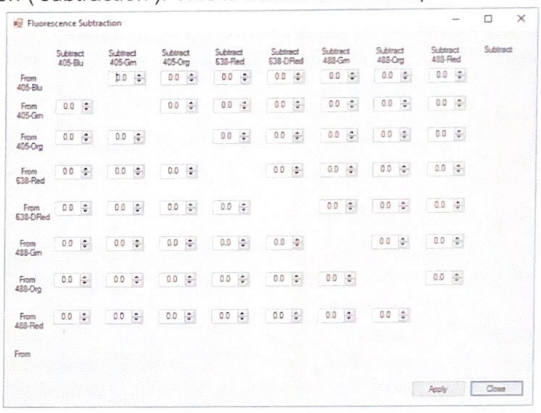
Events/ul and Events/sec average for all data in the memory buffer

Event rate stability monitor. This is the % difference between the average event rate at the current time, and the event rate during the first 30 seconds of the sample. Normal range -20% to +10%. An air bubble in the syringe can cause variation.

FIFO Full warnings. The number of FIFO overflows which are the normal cause of lost data due to the event rate being too high for the computer. Use thresholds to eliminated unwanted events to keep the event rate down and ensure no events are lost.

7.4.5.2 Subtraction (Fluorescence Compensation)

Fluorescence subtraction settings may be accessed using the button on the PMT tab of the Control Panel, or via the right click menu option on a cytogram with two fluorescence parameters. The dialog allows the setting of spectral overlap compensation ('subtraction'). This is useful when multiple fluorescent markers, with overlapping fluorescence emission spectra, are used.



Data in the buffer automatically 'replays' after changes to the subtraction values. Make sure the 'Apply Settings on Replay' option is ticked on the Gain tab (also accessible on the Setup menu).

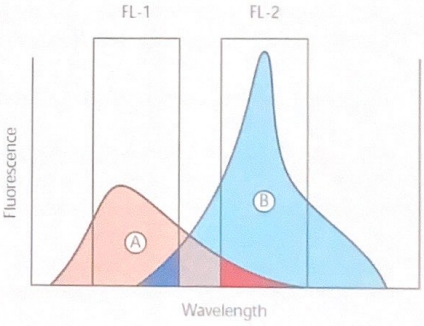
This dialog box can be left open while you test a variety of values (there's no need to close it each time you change a value).

The subtraction principles are the same as for any other flow cytometer:

1) Run a sample of cells or beads without fluorescent markers. Create fluorescence histograms gated on the light scatter cell/bead population. Position ROIs around the negative cells on the fluorescence histograms. Record the mean fluorescence intensities (MFIs) of the negative cell population.

2) Run the cell/bead sample labeled with one fluorochrome at a time (one sample for each fluorochrome) and save the data.

3) The fluorochrome emission should fall mainly in one fluorescence detector (this depends on the fluorochrome spectra and the fluorescence filters installed in your machine). Let's call this the '*fluorochrome detector*'.



4) For each single fluorochrome datafile, check the MFI of the cells on the other detectors (not the *fluorochrome detector*). If the MFI has increased it must be due to fluorescence spill-over from the fluorochrome, so adjust the appropriate control to subtract a percentage of the signal on the *fluorochrome detector* from each of the other channels where there is spill-over. You should end up with about the same MFI values as you recorded when no fluorescent label was used in step (1).

Note that the fluorescence subtraction values are dependent on photomultiplier voltages.

PROCEDURE

The user may be supplied with a set of datafiles to facilitate the compensation process, or they can create these template files themselves, for example:

COMP_UNLABELED.fcs
With cytograms or histograms for each fluorescence detector, gated on the cell population as measured by light scattering. Each cytogram should have an ROI which can be used to record the mean noise of the unlabeled cell population measured on each detector.

COMP_FITC.fcs
With cytograms displaying FITC on X axes against each other fluorescence detector on Y axis, with ROIs on the cell clusters to give the mean fluorescence from the Y parameters

COMP_PE.fcs
With cytograms displaying PE on X axes against each other fluorescence detector on Y axis, with ROIs on the cell clusters to give the mean fluorescence from the Y parameters

COMP_Cy5.fcs
With cytograms displaying Cy5 on X axes against each other fluorescence detector on Y axis, with ROIs on the cell clusters to give the mean fluorescence from the Y parameters

COMP_Cy7.fcs
With cytograms displaying Cy7 on X axes against each other fluorescence detector on Y axis, with ROIs on the cell clusters to give the mean fluorescence from the Y parameters

1) Load COMP_UNLABELED.fcs and Run unlabeled cells

2) Decide on PMT Voltages for the fluorescence detectors. These are typically simply the voltages stored in the template file. It is normal to keep the cell clusters below the 1000 position on a logarithmic axis (22 bit scale).

3) Record the mean unlabeled cell brightness (histogram bin) values for each detector. This is the Mean ROI statistic for the unlabeled cells.

4) Save the unlabeled cell data for future reference if desired.

5) Load or apply a settings file suitable for the color you want to compensate (e.g. COMP_FITC.fcs). Make sure the fluorescence PMT Voltages remain the same because the voltages affect the subtraction values.

6) Run a first sample labelled with only one fluorophore (e.g. FITC) and make sure the AUTOCOMP ROIs are well positioned to capture all cells

7) Save the data if desired.

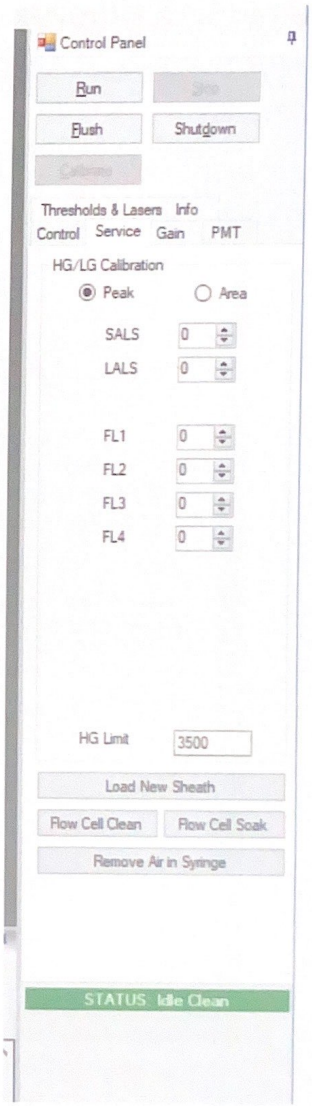
8) For each cytogram right click and choose 'Subtraction' to display the Subtraction window. Increase the subtraction until the MeanY statistic equals the noise value for that channel. The data will replay after each change to the subtraction value. Record the subtraction value found. Alternatively, for extra speed it may be possible to anticipate the amount of subtraction required because:

Beware of overcompensation which will put too many events against the axis because negative values due to overcompensation are all put into the first histogram bin.

9) Repeat for the other fluorophores by looping back to step (5) until all fluorophores are compensated

The subtraction settings are stored in the fcs file but beware that if you load another FCS file the subtraction values will be taken from that new file. To analyse a session's datafiles with new subtraction values, use the 'Load Data Only' option.

7.4.5.3 Service Tab



This dialog box is accessible only if the Level 1, 2 or 3 has been selected in the User Configuration software.

'Load New Sheath' pumps lots of sheath fluid through the system to clear any large air bubbles. It must be clicked whenever the machine detects and empty sheath tank.

'Remove Air in Syringe' is a useful button for occasional use (once a month). This moves the syringe piston slightly out of the precision glass syringe and allows sheath fluid to expel air from the syringe. Air in the syringe affects the accuracy of the sample system because the volume of trapped air changes a lot with pressure. The operator should perform this operation if they suspect inaccurate evts/ul readings. The button is enabled when the machine is connected to the histogram software and the machine is idle.

The "Flow Cell Clean" button draws cleaning fluid from the tank into the flow cell and sample tubing. This is automatically performed during the Shutdown cycle.

The "Flow Cell Soak" procedure should be used to address high light scatter PMT noise levels. It draws cleaning fluid into the flow cell and leaves it there for approximately 10 minutes.

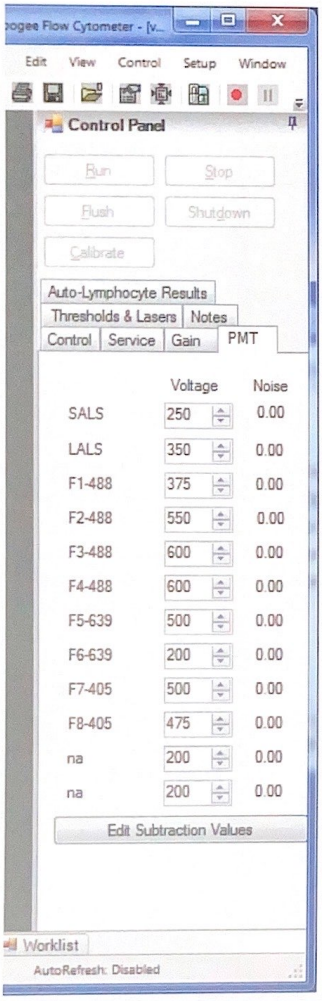
The following controls are not normally used:

The High/Low channel calibration settings should be set to 0 for normal use. These settings may be used to ensure no gap or overlap in the data at the High Gain Cutoff position on the histogram. It can be set between -1500 and +1500 (65535 resolution).

The HG Limit control allow adjustment of the histogram channel number (65535 resolution) at which the data acquisition electronics switches from a high gain signal to a lower gain signal. This should be set to approximately 3500 under normal circumstances, but can be changed to lower values if there is an imperfection in the data at channel 3500 (e.g. a gap or overlap in data) to move the imperfection away from data of interest.

7.4.5.4 PMT Voltage Tab

The PMT tab is used to adjust the photomultiplier tube (PMT) voltages. For Auto-40 machines and Stand-Alone machines, these voltages are automatically set by the machine during a calibration and should not be altered by the user (changing them will set the machine to 'Uncalibrated')



Increasing the PMT voltage increases the number of electrons emitted by the tube for each photon received. i.e. the gain of the PMT tube increases when you increase the PMT voltage.
Larger signals are plotted further to the right on histograms, so to move a population of cells to the right, increase the PMT voltage.

The MiniFCM can be used to measure a very wide range of particles. If measuring bright particles (particles which scatter a lot of light or which emit a lot of fluorescence), then the PMT voltage may be set as low as 200Volts. If measuring very dim particles, the PMT voltage will need to be set higher.
As a general guide, if the noise level rises above 0.5 then reduce the PMT voltage and use gain instead. This is particularly applicable to the scatter PMT voltages because the scatter PMTs receive a lot of background light.

The background light (noise) levels reaching the fluorescence PMTs are generally very low because the background is eliminated by the fluorescence filters. It is important to keep all the noise levels to below 1.0. Lowering the PMT voltage lowers the noise.

As a guide, the fluorescence PMT voltages are typically set in the range 350 to 750 Volts.
The scatter PMT voltages are typically set in the range 250 to 450 Volts.

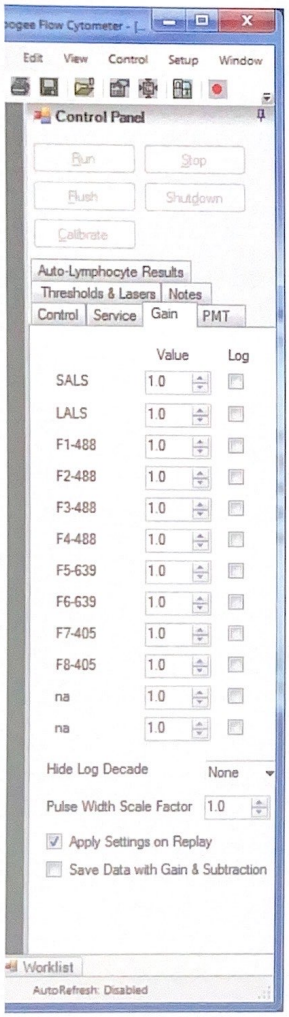
If the scatter PMT noise levels are greater than 1.00 when 1um calibration particles are in histogram channel 20000/65535, with gain 1, then perform a Flow Cell Soak operation, because this is an indication of a dirty flow cell.

If the noise levels rise above 1.00 the response of the PMT tube may become non-linear and unpredictable threshold triggering problems may occur, giving misleading data. The machine should not be used in this condition (perform a Flow Cell Soak, lower the PMT voltage or have the machine serviced).

See the 'Subtraction' section above for a description of the subtraction dialog accessible by clicking the button on this 'PMT' tab.

7.4.5.5 Gain Tab

The Gain tab allows setting of the linear and logarithmic gains. These gains are applied to data from the corresponding PMT tube as it arrives in the histogram software.



To enable the logarithmic gain controls it is necessary to click the 'Log Gain' option (which applies to all data in that channel as it arrives) on the 'View' pull-down menu.

When using logarithmic gain, if the lower (left hand side) one or two decades contain no useful data, you should reduce the PMT voltage. Alternatively you can choose to hide the first and/or second decades.

The gain settings can be used to apply post analysis gain to data – simply load the data, check "Apply settings on replay", set the desired gains (and fluorescence subtraction, if desired) and click 'Replay' on the Toolbar.

'Apply Settings on Replay' applies the current gain and subtraction settings to the current dataset (buffer) when you click the Replay button on the toolbar or select the Replay option on the Control menu. The actual data buffer is not modified, but data from the current buffer is adjusted before being displayed on the screen.

The 'Save Data with Gain and Subtraction' option determines whether the current data buffer is saved before or after gain and subtraction settings have been applied.

So the user may choose between the following typically modes of operation:

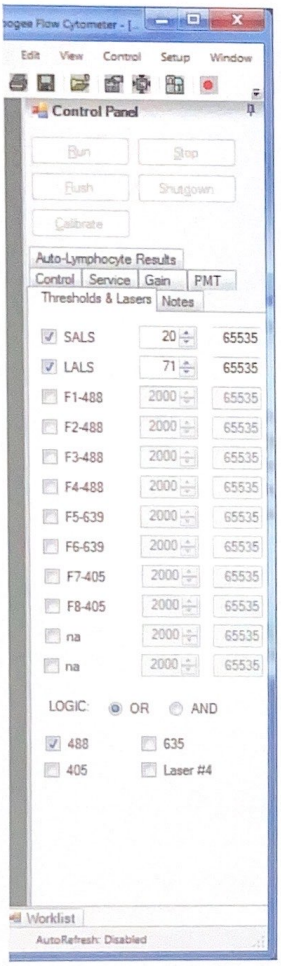
1. Select 'Apply Settings on Replay' and deselect 'Apply Settings to Stored Data'.
When the data buffer is saved to a file, the data is stored in the file before any Gain or Subtraction has been applied. Gain and Subtraction can still be used while running samples, and Gain and Subtraction will be applied upon replay, but the machine will store the raw data when you choose 'Save'. This makes it simple to change Gain and Subtraction settings after running samples.

OR

2. Select 'Apply Settings to Stored Data' and deselect 'Apply Settings on Replay'.
When the data buffer is stored to a file, the data is stored after Gain and Subtraction have been applied. In this case you must deselect 'Apply Settings on Replay' to ensure the gain and subtraction settings do not get applied a second time when the data is replayed or loaded from the saved file.

7.4.5.6 Thresholds & Lasers Tab

Trigger thresholds are used to eliminate optical noise events and unwanted particles (e.g. debris) in the sample so that processing power is reserved for the particles of interest.



The thresholds may be set by dragging the dotted red line on the histogram, or by typing threshold levels directly into the boxes on the Threshold tab.

The values displayed are on a 16 bit scale (i.e. 1 to 65535).

If thresholds are active on more than one PMT detector, the combination operator applies.
For example, if the AND operator is selected then a particle must give signals greater than the threshold levels for all PMTs with active thresholds, in order for it to be counted.
If the OR operator is selected, the particle must give a signal greater than the threshold level on any one of the PMTs in order for it to be counted.

The trigger thresholds act on the pulse heights (not pulse integral (Area)).

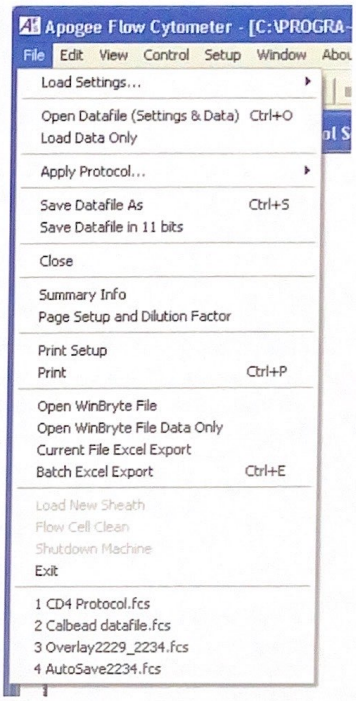
CAUTION: Avoid using very low (e.g. less than 500) scatter thresholds unless the PMT noise level (see PMT tab) is low (e.g. less than 0.5). If a very low threshold is used on a channel with a high noise level the trigger circuit may not function correctly.

CAUTION: If using AND logic, do not set any of the threshold at a level where there would be in the instrument noise because the noise will interfere with the triggering circuit. As a guide, when using AND logic, keep the thresholds above channel 500 on light scatter and above channel 200 on fluorescence.

An upper trigger threshold may also be set. The upper threshold is disabled when set to 65535, and the upper threshold logic is always AND. In other words if any event is above any enabled upper threshold, it will be rejected.

Depending on the types of laser installed, the lasers may turn on/off immediately in response to the check boxes, or may require several minutes of warm-up time.

7.4.6 The 'File' menu



7.4.6.1 Load Settings…

Frequently used template files may be saved to folder:

C:\Program Files (x86)\Apogee\Protocols

Files in this location will appear on the "Load Settings…" menu and are common to all users. New files will not appear until the software has been restarted.

Auto40 cytometers have the files in

C:\Program Files\Apogee\SampleData

And they are typically:

CD4 Protocol.fcs - CD4 Settings
CD4 Percentage Protocol.fcs - CD4-45% Settings
CD4 Percentage Manual Protocol.fcs - Manual CD4-45% Settings

7.4.6.2 Opening a data file

To load a previously saved data file, click on the File menu and choose 'Open Data file (Settings & Data)' or click on the Open icon () on the toolbar.

Each time a saved data file is opened, the instrument settings and page layout are reloaded (except the sample volume and Flush count). To avoid setting and page layout changes choose the 'Open Data Only' option instead.

7.4.6.3 Loading Data Only

To load only the data from a stored file, without changing the instrument settings and graphs, the "Load Data Only" option can be used. This menu option overwrites any data currently displayed. Note that the PMT Voltage settings affect the gain of the photomultiplier optical detectors and so cannot be changed after data acquisition. Similarly the threshold levels act on the signals from the photomultiplier tubes so cannot be changed after data acquisition. Clearly the sample delivery rate and sheath pressures also cannot be changed after data acquisition.
However, many other settings are pure software settings. For example, software gain and datagram settings can all be changed after data acquisition because they do not involve the electronic hardware.

The 'Load Data Only' option allows you to view any datafile with the current software settings.

7.4.6.4 Apply Protocol

This menu option overwrites the software settings with those from a stored file, but preserves the data in the current buffer. The files used are stored in

C:\Program Files (x86)\Apogee\Protocols

and can be overwritten if required.

7.4.6.5 Saving a data file

During sample analysis or after sample analysis has been stopped, it is possible to save the data file by selecting 'Save Datafile As' from the File pull-down menu. Alternatively click the Save icon () on the toolbar. Often the AutoSave option accessible via the WorkList header is the best way to save the data from each sample.

7.4.6.6 Adding sample information to the data file

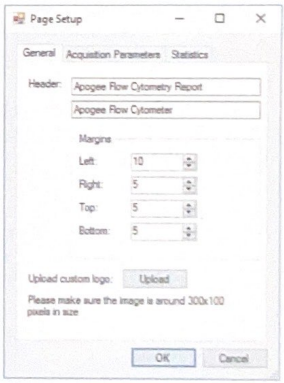
If you wish to add information to the acquired data, click on the File menu and select the option 'Summary Info' from the drop-down menu. The Summary information dialog box appears. These notes are included in the printed report, and are stored in the data file.

Note that after entering information here, the next sample run will inherit the same information.

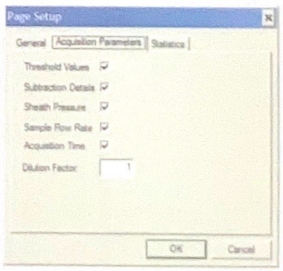
Clicking on the  icon also opens the Summary Info dialog box.

7.4.6.7 Page set-up

The page layout for printed reports can be set by clicking the 'Page Setup' option from the File menu, or by clicking the  icon.



On the General tab you can set the page margins, and header text.

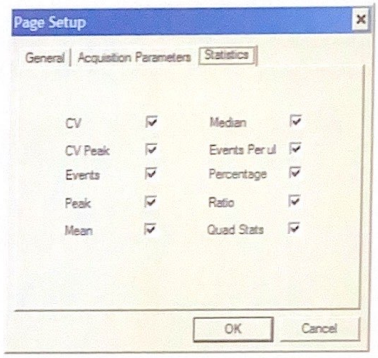


On the Acquisition Parameters tab you can choose which instrument settings to include in printed reports.

You can also set the dilution factor which is used to correct the evts/ul readings to account for dilution during sample preparation.

For example, if a blood sample has been diluted by a factor of 10 during sample preparation you can enter a Dilution Factor of '10' in order to correct the evts/ul

readings so that the readings of the pure blood are displayed.
In other words, with a dilution factor of 10, the software will indicate 10 times more events per ul than the machine actually counts.



On the Statistics tab you can choose which statistics to display on the printout. By default all the statistics are selected.

7.4.6.8 Print set-up

To open the Print set-up dialog box, click on the File menu and select the Print Set-up option from the drop-down menu. Standard Windows options are offered.

7.4.6.9 Print

To print a document click on the File menu and select Print from the drop-down menu.
If there is no need to edit printer settings, simply click on the Print icon on the toolbar.

7.4.6.10 Current File Excel Export

Sends current data and settings to MS Excel. If no Excel spreadsheet is open, it will create a new spreadsheet, otherwise it will add the data to the currently open spreadsheet. By clicking this option once for each datafile a spreadsheet can be created to allow comparison of ROI statistics from multiple files. Requires MS Excel to be installed.

7.4.6.11 Batch Excel Export

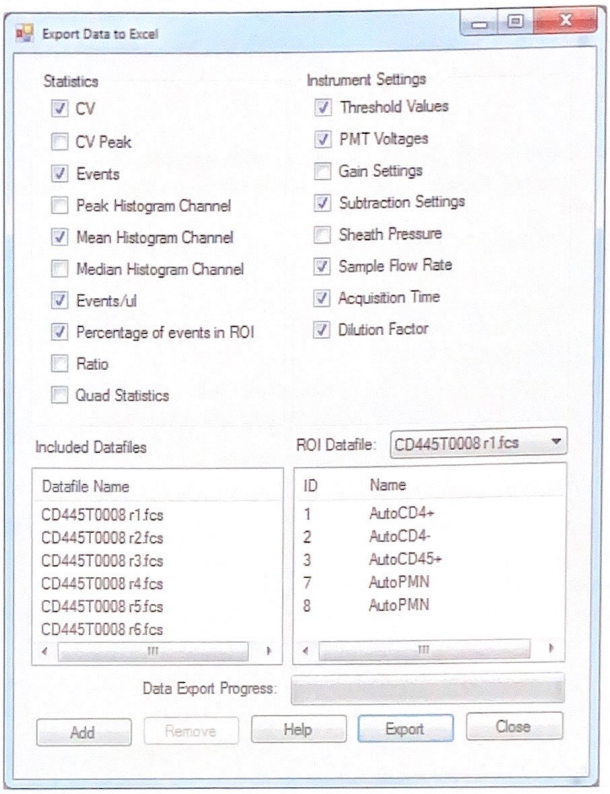
The Excel Export module allows statistics from a batch of data files to be sent to Microsoft Excel ™.

The module works as follows:

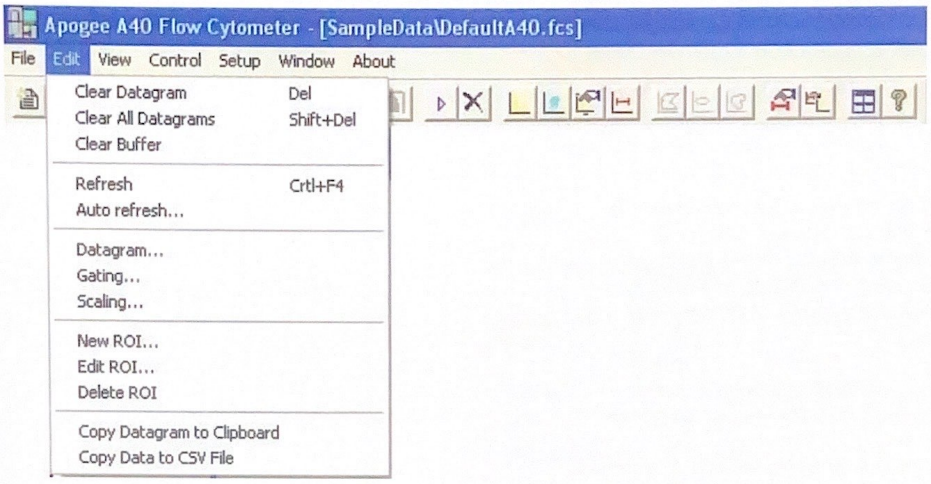
- The user clicks the 'Add' button to select one or more (use *Shift* or *Ctrl* keys) data files.
- The user chooses ONE of the data files from which the software will take ROI position information. This is called the "ROI Data file" and the ROI settings from this file will be used to analyze all files in the list.
- When the user then selects (highlights) which ROI(s) to display in Excel, the Export button becomes enabled.

For example, if using this module to obtain a report showing the number of bacteria in a batch of samples, each file in the set of data files must have the population of bacteria in the roughly the same place on the histograms so that the same ROI settings file can be used for all files in the list.

Requires MS Excel to be installed.



7.4.7 The 'Edit' menu



7.4.7.1 Clear Datagram

This function clears the data in the active histogram or cytogram, without removing data from the buffer. 'Replay' can be selected to recover the data

7.4.7.2 Clear all Datagrams

This function clears the data in all the open histograms and cytograms, without removing the data from the buffer. 'Replay' can be selected to recover the data.

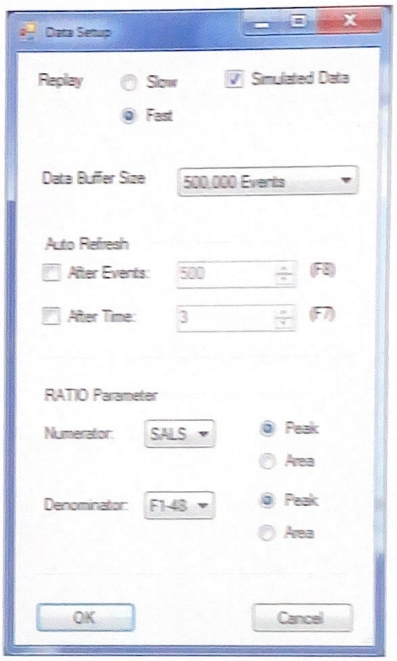
7.4.7.3 Clear buffer

Clicking on this command all the data will be removed from the histograms and cytograms and from the PC memory buffer. 'Replay' cannot then be selected to recover the data.

7.4.7.4 Refresh

Clicking on this command or on the  icon, all the data will be removed from the histograms and cytograms, and from the memory buffer.

7.4.7.5 Data Setup Dialog



The auto-refresh option automatically clears the screen and buffer after specified intervals. This is particularly useful when making adjustments to settings such as PMT voltages and gains.

Clicking on the Auto Refresh option opens the List Data Set-up dialog box.

Click on this arrow to select the maximum buffer size. When the data buffer is full, the instrument stops collecting data and the user can choose between clearing the buffer to continue sample analysis, and stopping the sample (but keeping the contents of the current data buffer). A large buffer requires a lot of PC memory (RAM).
The simulated data check box can be used to create simulated data. To see the real data, deselect this check box.

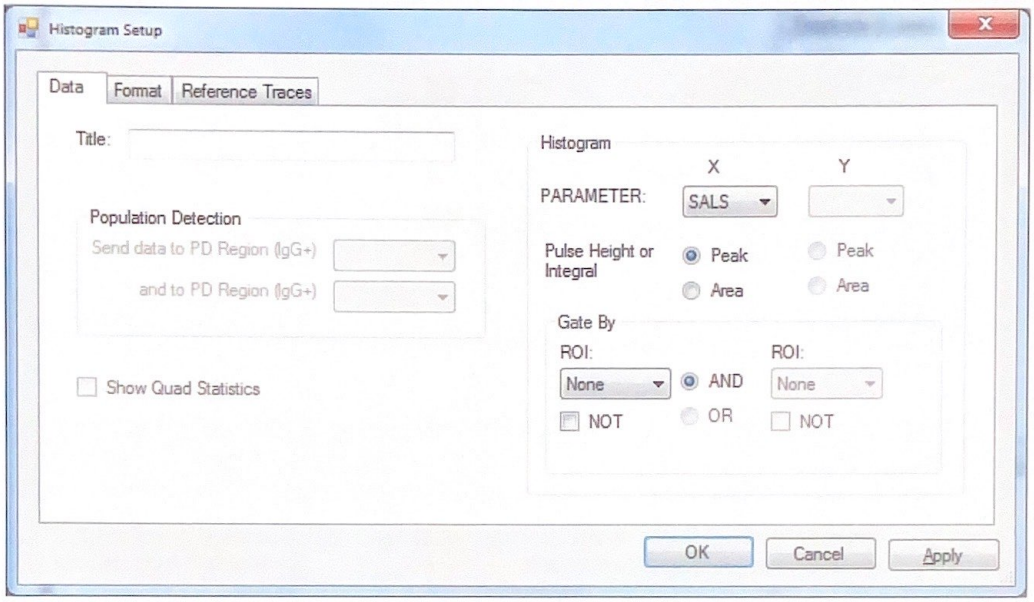
The RATIO parameter can be plotted on any histogram as if is a signal from one PMT. It can be defined here.

7.4.7.6 Datagram

By clicking on this command or on the  icon, the histogram set-up dialog opens for the currently selected histogram or cytogram (dual parameter histogram). The same window can be opened by double clicking the histogram/cytogram or right clicking on the histogram/cytogram and selecting Edit datagram from the context menu.
Depending upon whether you are configuring a histogram or cytogram, the options are different. For this reason, the two dialog boxes will be described separately.

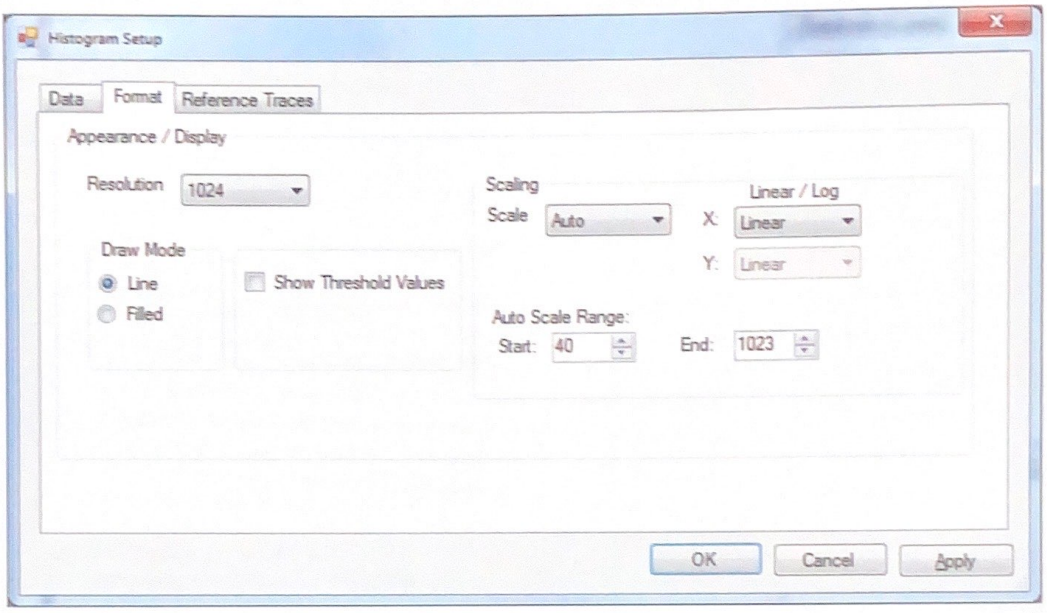
7.4.7.7 Histogram Set-up

7.4.7.7.1 Data Tab



Title: write a description for the histogram;
Parameter: click on the arrow to select the acquisition parameter;
Peak/Area: choose whether to display pulse height ('peak') or pulse integral ('area') data;
Channel resolution: click on the arrow to select the channel resolution (between 32 and 65536);
Gating: click on the arrow near to ROI text box on the left to select any region of interest to be used to gate the data. If more than 1 region of interest is to be used for gating, choose a Boolean operator. Tick the box to gate by events NOT in the selected region.

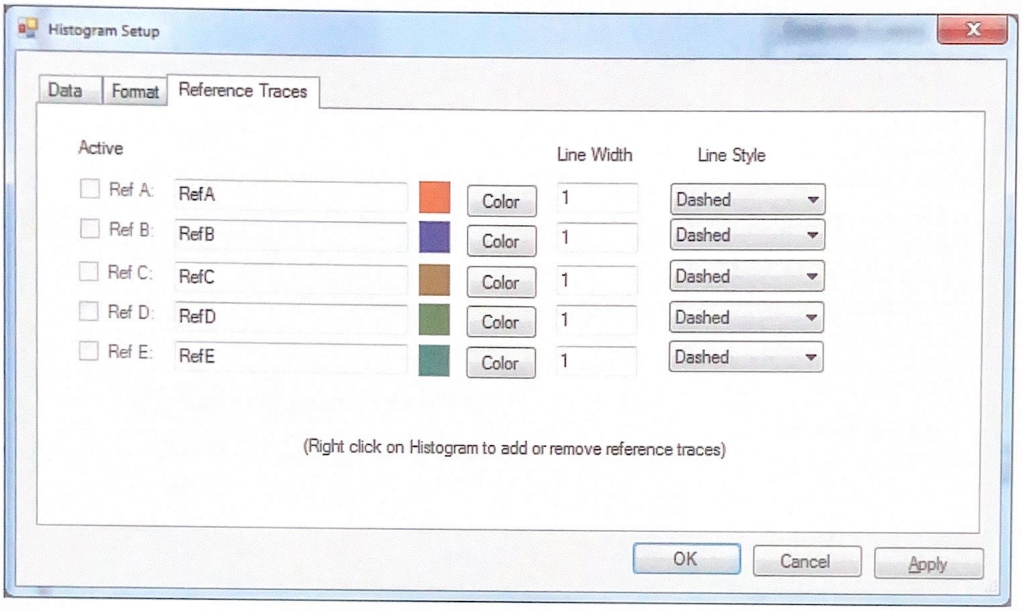
7.4.7.7.2 *Format tab*



Draw mode: Choose between line and filled modes;
Threshold: click on the check box to display the threshold value on the histogram;

Scale: Select the vertical scaling (number of events per histogram bin); if Auto is selected, the histogram y axis will scale automatically to the histogram channel with the most events inside the Auto Scale range specified.
Auto Scale channels: insert into the text boxes the starting and ending point for the Autoscale.
X: click on the arrow to select the x axis mode. Linear or logarithmic options are available. When plotting a logarithmic scale, you can choose to hide the first one or two decades. So the log scale can be plotted with 4.8 ("Log – full"), 3.8 ("Log – 1st") or 2.8 ("Log – 2nd") decades.

7.4.7.7.3 *References Tab*



This self-explanatory menu can be used to configure the draw mode for reference traces saved by right clicking on a histogram.

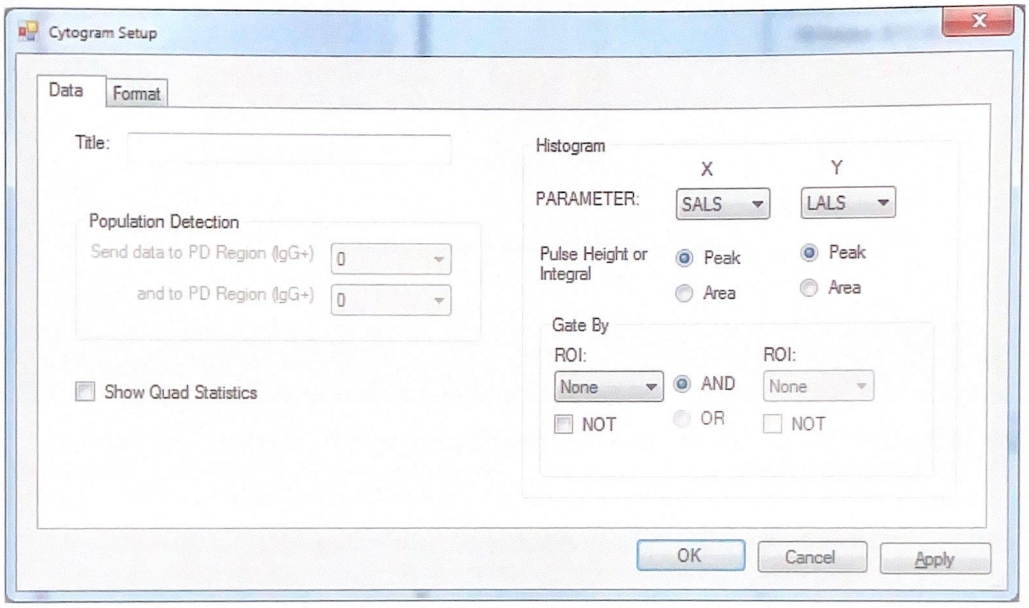
Histogram data from multiple files can be overlaid by the following steps:

- With data for the first histogram trace displayed, right click on a histogram and save the current data trace as a reference.
- Load the second data set that you want to compare using the 'Load Data Only' option on the file menu.
- The previous reference trace and all graphs will be maintained, but the new data will be played.
- You can now save this second data set as another reference trace.
- Repeat to overlay up to 5 datasets
- Saving the file will also save the reference traces

7.4.7.8 Cytogram set-up

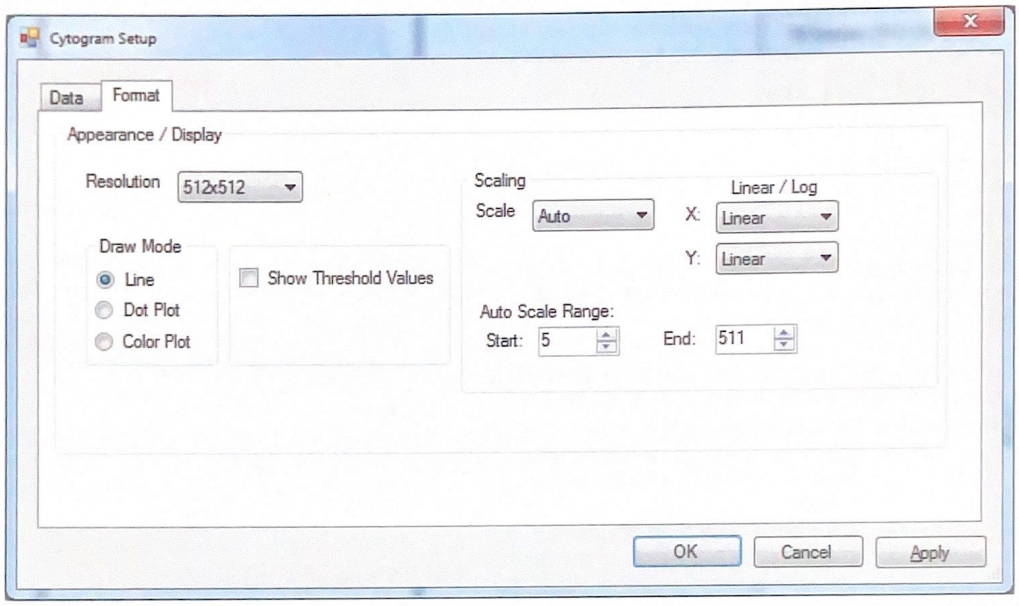
7.4.7.8.1 Data Tab

Title: You may enter a description for the cytogram.
Parameter: choose the two acquisition parameters;
Peak/Area: choose whether to display pulse height ('peak') or pulse integral ('area') data;



Gating: click on the arrow near to ROI text box on the left to select any region of interest to be used to gate the data. If more than 1 region of interest is to be used, choose a Boolean operator.
Show Quad Statistics: select this check box to draw quadrants on the cytogram.

7.4.7.8.2 *Format Tab*



Channel resolution: click on the arrow to select the channel resolution (between 32 and 1024);
Draw mode: click on the selection box to choose between line, dot plot and colour dot;
Threshold: click on the check box to see the threshold value on the histogram;
Scale: click on the arrow to select the end of scale of the z axis (frequency); if Auto is selected, the cytogram z axis will update automatically to contain all the events. Within the Auto Scale Range.
Auto Scale channels: insert into the text boxes the starting and ending point for the Autoscale.
X and Y: click on the arrows to select the x axis mode (linear or logarithmic with 4.8, 3.8 or 2.8 decades);

7.4.7.9 Gating

Click the  icon or select 'Gating' from the 'Edit' menu to view the Histogram Setup menu on which you can specify gating rules for the current histogram, e.g. 'only show particles which fall inside region of interest number 3'.

7.4.7.10 Scaling

Adjust the scaling of the current histogram or cytogram by opening the Histogram set-up or the Cytogram set-up dialog box (see above). Alternatively use the controls at the top left of the datagram window.

7.4.7.11 ROI Dialog

The ROI dialog offers various statistical and format options. It can be accessed by clicking one of the toolbar icons:

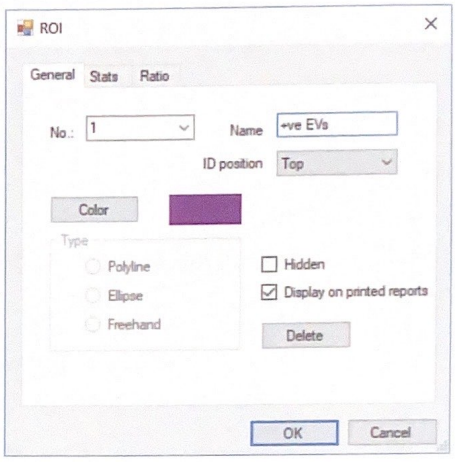
On the General tab it is possible to set the name and the colour of the ROI. In case of cytogram it is also possible to select the type of ROI to be drawn: polygonal, elliptical or freehand.
The statistics are written using the chosen ROI colour, so avoid the use of very light colours that may be difficult to read on printed reports.

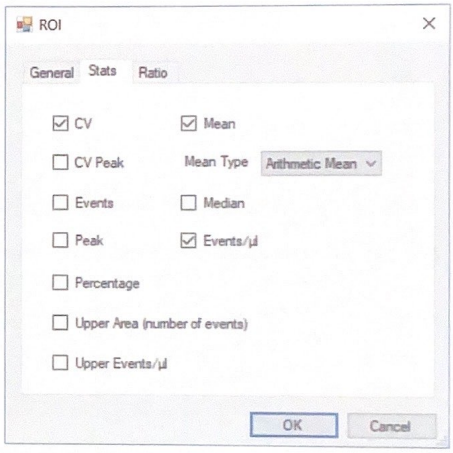
On the Stats tab it is possible to select the statistics shown at the bottom of the datagram. In case of histogram there are more possibilities:

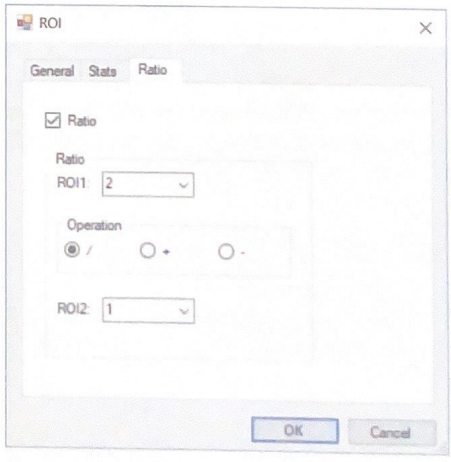
- CV = coefficient of variation (100x standard deviation divided by the mean);
- CV Peak= coefficient of variation calculated at half height of the peak;
- Events= number of events inside the ROI;
- Peak= modal channel of the population in the ROI;
- Mean= arithmetic or geometric mean histogram bin of the population;
- Median= median histogram bin of the population in the ROI;
- Events/µl= number of events (particles) in the ROI per microliter of sample;
- Percentage= percentage of the events inside the ROI compared with the total number of events.

For cytograms the options are limited to Events, Events/µl and Percentage.

The Ratio tab gives to the user the possibility to set an arithmetic calculation between two different ROI.
To do that, click on the Ratio check box to activate, click on the arrow close to the ROI1 text box to select the first region of interest, select the mathematical operator to be used by clicking on the opposite check box and finally click on the arrow close to the ROI2 text box to select the second region of interest.







7.4.7.12 Edit ROI

This command opens the ROI dialog box (see above) and allows the user to change any settings. It can be used only if the ROI has been already drawn. The same window is available by clicking the  icon.

7.4.7.13 Delete ROI

This command deletes the currently selected ROI (select and ROI by clicking on it). The currently selected ROI has its data displayed at the bottom of the histogram window.

7.4.7.14 Copy datagram to Clipboard

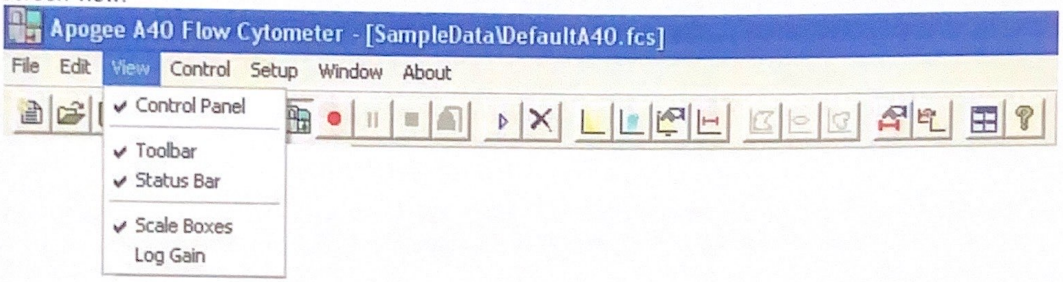
This command copies the selected datagram to the Windows Clipboard, to be used with other graphics programs. A datagram can be embedded in a text file or in an Excel worksheet, or pasted in a graphic program (e.g. Windows 'Paint').

7.4.7.15 Copy datagram to CSV file

This save data from the currently selected histogram as a Comma Separated Values (.csv) file which can be read by programs such as Microsoft Excel. Data is stored as histogram channel number versus number of events in the channel, so graphs can be plotted easily from the .csv file using third party software.

7.4.8 The 'View' Menu

This menu allows sections of the screen to be hidden. In this way it is possible to customize the screen view.



7.4.8.1 Control Panel

Toggles the visibility of the Control Panel on the right part of the screen. If not selected, the histograms and cytograms will fill the work area of the screen. Alternatively it is possible to click on the toolbar icon : 

7.4.8.2 Toolbar

Toggles the visibility of the toolbar on the upper part of the screen.

7.4.8.3 Status bar

This toggles the visibility of the status bar on the lower part of the screen.

7.4.8.4 Scale boxes

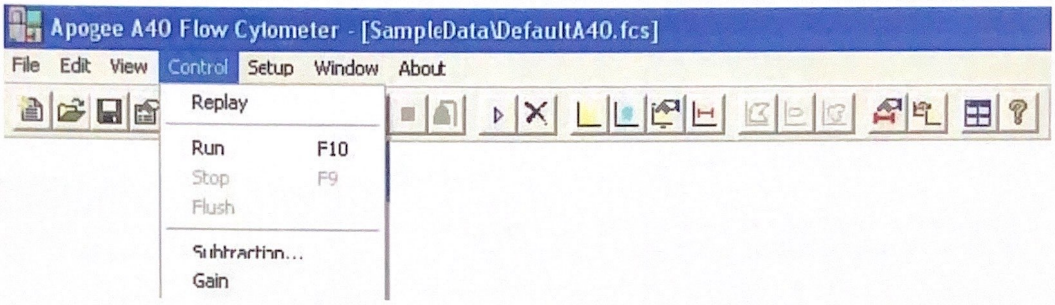
Allows to hide or show the scale boxes on all the histograms and cytograms on the screen.

7.4.8.5 Log Gain

Activates the log gain check boxes on the Control Panel Gain tab.

7.4.9 The 'Control' Menu

This menu contains commands, most of which are also present on the Control Panel.



7.4.9.1 Replay

Alternative click on the  icon on the toolbar. This button replays data in the current data buffer. After changing gating of gain settings, replay the data for the new settings to take effect.

7.4.9.2 Run

Runs a sample. Normally this is not required because samples automatically run when the sample arm is moved into the run position. Alternatively click on the  icon.

7.4.9.3 Stop

Stops a currently running sample. Alternatively click on the  icon.

7.4.9.4 Flush

Flushes the sample tubes. Make sure the sample arm is in the flush position first. This button is not normally required because the machine automatically flushes when the sample arm is moved to the flush position. Alternatively click the  icon.

7.4.9.5 Subtraction

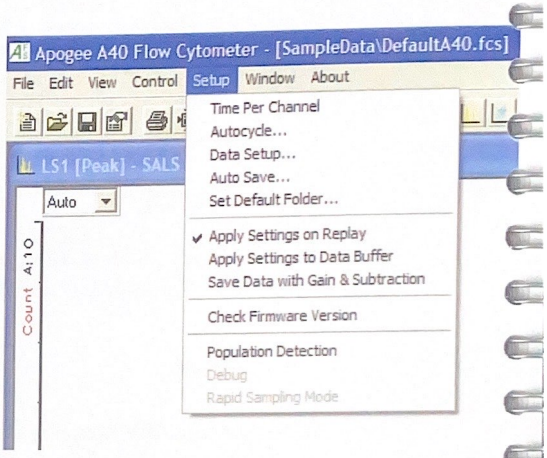
For compensating for spectral overlap when multiple fluorescent markers are used. This command takes you to the Subtraction window; also accessible via the PMT tab on the Control Panel.
See section 7.3.5.2 for further information.

7.4.9.6 Gain

This command takes you to the Gain tab of the Control Panel. Software gain is applied to data as it is collected, but you can choose to save the data without the gain settings (see checkbox on Control Panel). These gain settings can also be used to apply post analysis gain to data, but first select "Apply settings on replay" from the settings menu or check the box on the Gain tab of the Control Panel.

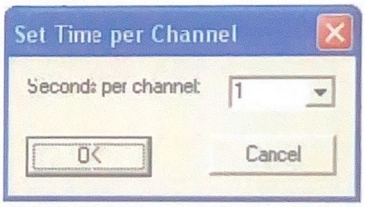
7.4.10 The 'Setup' Menu

This menu contains several commands which do not appear in other windows.

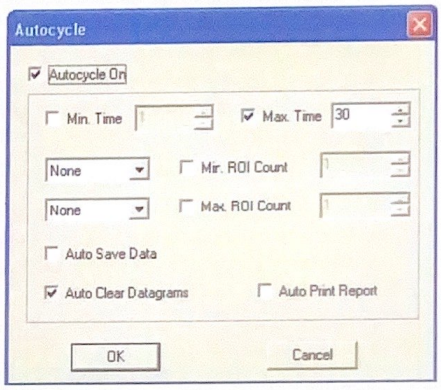


7.4.10.1 Time per channel

Adjusts the time interval per histogram channel. This applies to histograms with time selected as one of the axes. Time can be selected from the Histogram set-up menu which can be accessed by double clicking the histogram. For example, this is useful if you would like to plot a graph of cell fluorescence versus time.

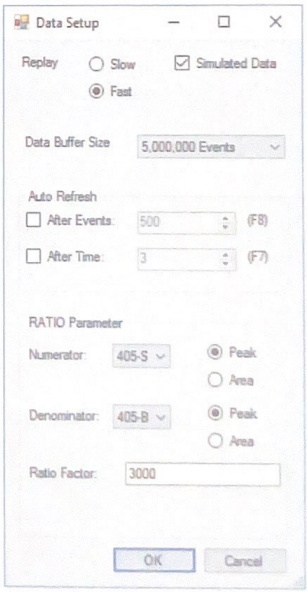


7.4.10.2 Autocycle



For automatically stopping samples after specified numbers of events or after a specified time interval. Click on the arrows near to the text boxes to select the minimum or maximum number of seconds of acquisition or, if preferred, the minimum or maximum number of events to be acquired inside a specified ROI.
Select post acquisition operations to automate the job.
To activate the Autocycle, make sure the 'Autocycle On' checkbox is checked.

7.4.10.3 Data Setup



The data buffer size allocates computer memory for temporarily storing the data. If the buffer size is large and the PC has less than 512MB RAM, the PC will run slowly.

Auto-refresh options are also available from this menu. Auto Refresh automatically clears the screen and buffer after specified intervals. This is particularly useful to service engineers when aligning the machine.

When the data buffer is full, the instrument stops collecting data and the user can choose between clearing the buffer to continue sample analysis, and stopping the sample (but keeping the contents of the current data buffer).

The simulated data check box can be used to create simulated data. To see data from the FCM Control program deselect this check box.

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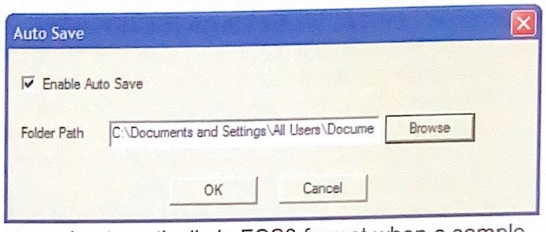
7.4.10.4 Auto Save

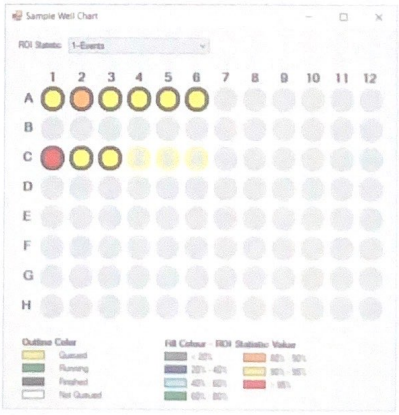


If running a batch of many samples, it is convenient for the data to be saved automatically. SampleIDs entered in the WorkList are used for the file name, or an automatically incrementing file name is issued.

When this option is enabled, the data and settings are saved automatically in FCS3 format when a sample stops. If the sample was Queued in the WorkList then the SampleID will be used as the file name and the Sample Notes will be set to the 'Description' in the WorkList:

With these WorkList settings, when instructed to Run, the machine will take a sample from well A1 (96 well plate) and save the data when the sample stops with the file name "Buffer Control.fcs" in folder "C:\Temp".

After 1000 files have been collected in the specified folder, the oldest file is deleted when a new file is saved to avoid filling up the hard disk. Specify a different folder before this limit is reached to avoid losing the old data.



A 'View Plate' button is in the WorkList header for software versions after 255.0.0.158. This offers the following features:

- Click on a well to add it to the WorkList.
- Double click a well to add it a second time to the WorkList if you want it to be run twice.
- Add an ROI statistic to the WorkList and then select the statistic in the Plate View to colour the wells according to the value of the statistic.

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7.4.10.5 Set Default Folder

Allows the user to change the default file load and save folders for fast and convenient file management.

7.4.10.6 Apply Settings on Replay

When this is selected, gain settings and subtraction settings are applied to the current data buffer when 'Replay' is selected. The data file is not modified and if saved after applying different settings, no changes will have been made to the data.

7.4.10.7 Apply Settings to Loaded Data (Apply Settings to Data Buffer)

To enable this option, 'Apply Settings on Replay' must be checked/active. Select this menu option to apply the current gain and subtraction settings to the current data buffer. The current data buffer will be permanently modified and the subtraction and gain settings will be reset to their default values (0 for the subtraction controls and 1 for the gain controls). Use this option with caution because there will be no record that you have modified the data in this way. It is more common to store the data without gain and subtraction, and to apply the gain and subtraction settings on replay rather than permanently applying the settings to the data with this menu option.

7.4.10.8 Save Data with Gain and Subtraction

The 'Save Data with Gain and Subtraction' option determines whether, when the current data buffer is saved to a file, data is saved before or after gain and subtraction settings have been applied.

Users may choose between the following typically modes of operation:

1. Select 'Apply Settings on Replay' and deselect 'Apply Settings to Stored Data'.
When the data buffer is saved to a file, the data is stored in the file before any Gain or Subtraction has been applied. Gain and Subtraction can still be used while running samples, and Gain and Subtraction will be applied upon replay, but the machine will store the raw data when you choose 'Save'. This makes it simple to change Gain and Subtraction settings after running samples.

OR

2. Select 'Apply Settings to Stored Data' and deselect 'Apply Settings on Replay'.
When the data buffer is stored to a file, the data is stored after Gain and Subtraction have been applied. In this case you must deselect 'Apply Settings on Replay' to ensure the gain and subtraction settings do not get applied a second time when the data is replayed or loaded from the saved file.

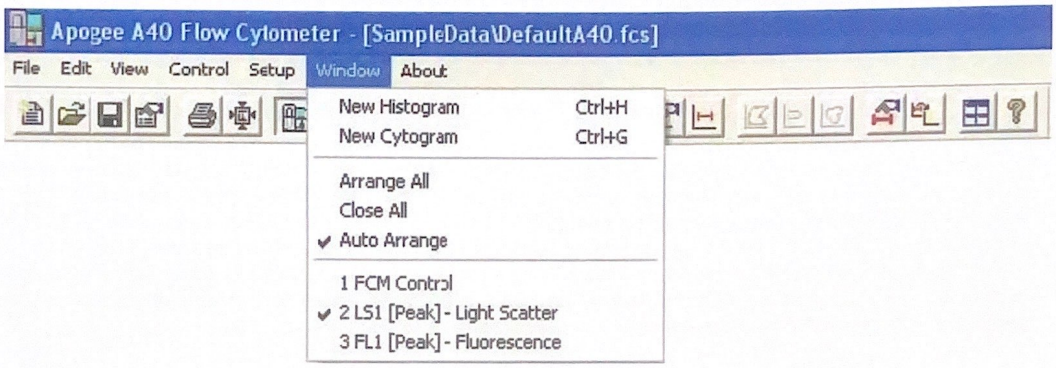
7.4.10.9 Check Firmware Version

This option is not normally used. It causes the histogram software to check the FCM Control version next time the histogram software starts. If communication with the machine fails, it may be necessary to click this option to recover full operation. Each time you run the histogram software, when the histogram software connects to the FCM Control program, a splash dialog is displayed giving the FCM Control ('firmware') version detected. The current FCM Control version is also displayed on the top line of the MiniFCM's display.

Make sure the firmware FCM Control version detected upon connection, agrees with the version displayed on the MiniFCM display, if not, select this menu option and restart the histogram software

7.4.11 The 'Window' Menu

These options are self explanatory. The window management system allows easy changes between tiled and tabbed display modes via toolbar icons.



7.4.11.1 New Histogram

Opens a new histogram on the working space.

7.4.11.2 New Cytogram

Opens a new cytogram on the working space.

7.4.11.3 Arrange All

Arranges all the opened datagrams in the work space. Alternatively click the  icon.

7.4.11.4 Close All

Close all the opened datagrams in the work space.

7.4.11.5 Auto Arrange

If checked, the histogram and cytogram windows get arranged automatically.

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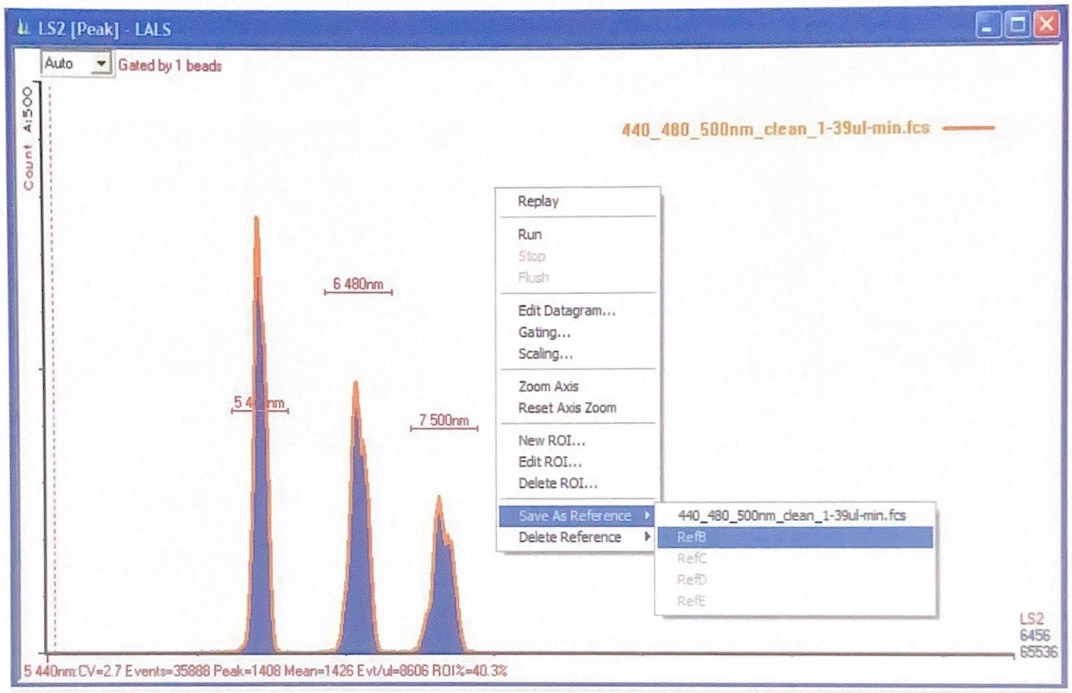
7.4.12 The 'About' Menu

Shows the installed software version and copyright information.

7.4.13 Histogram & Cytogram Context Menu (right click)



The context menu is available by right clicking on a histogram or cytogram. It offers functions available on other menus plus an option to save and delete reference traces. The draw mode and description of the reference traces can be edited using the histogram setup menu. The reference trace feature is available for histograms only. The number of references is limited to two for very high resolution histograms.

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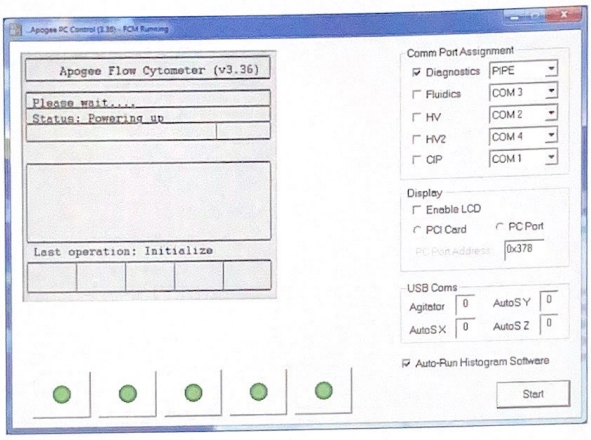
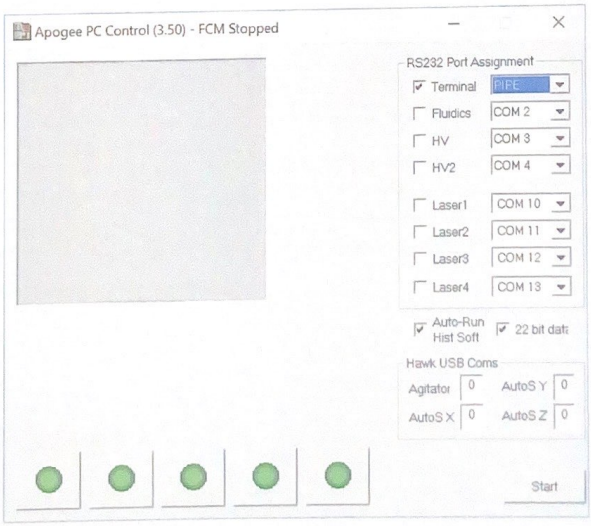
7.5 The 'FCM Control' Software

The FCM Control software controls and receives data from the hardware. It runs on the computer inside the cytometer. This program runs automatically in the background when Windows starts and requires no user interaction. When logged in without administrator permissions, the FCM Control program may not be visible in the Task Manager.

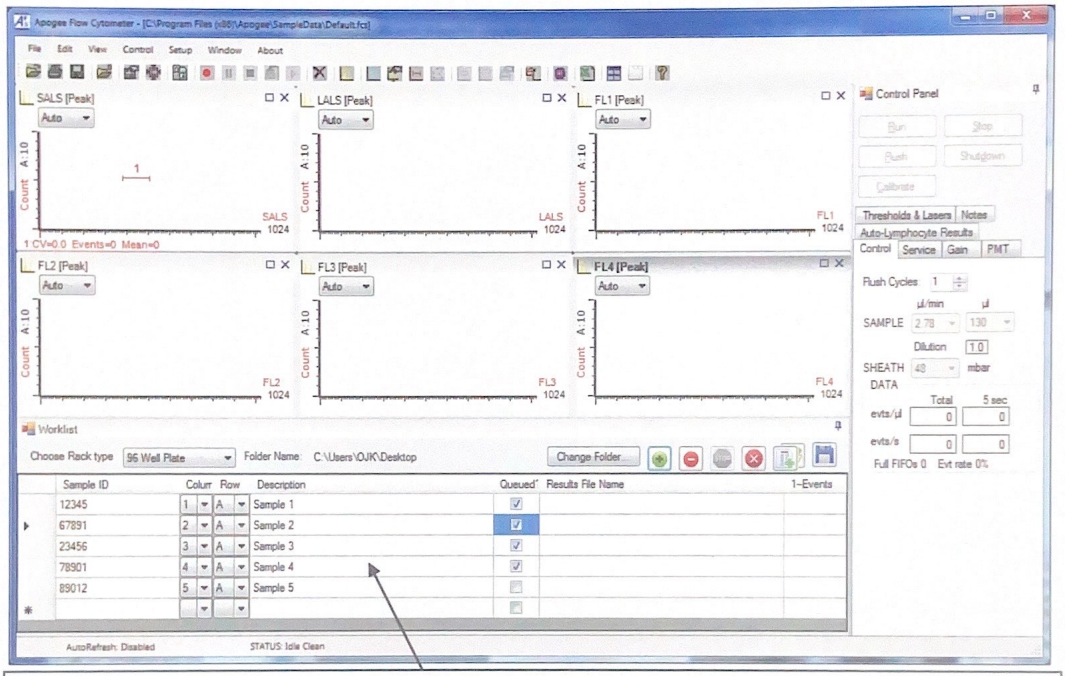




7.6 Work List Functionality (Histogram application)

The Work List option facilitates sample management and also data analysis. It is required for systems fitted with an AutoSampler but can also be used when running samples manually as it is a means by which to enter information about a group of samples you plan to analyse and offers a means to compare data from multiple samples.

You can enter a list of samples (or open a worklist created in csv format) and then run them one by one:

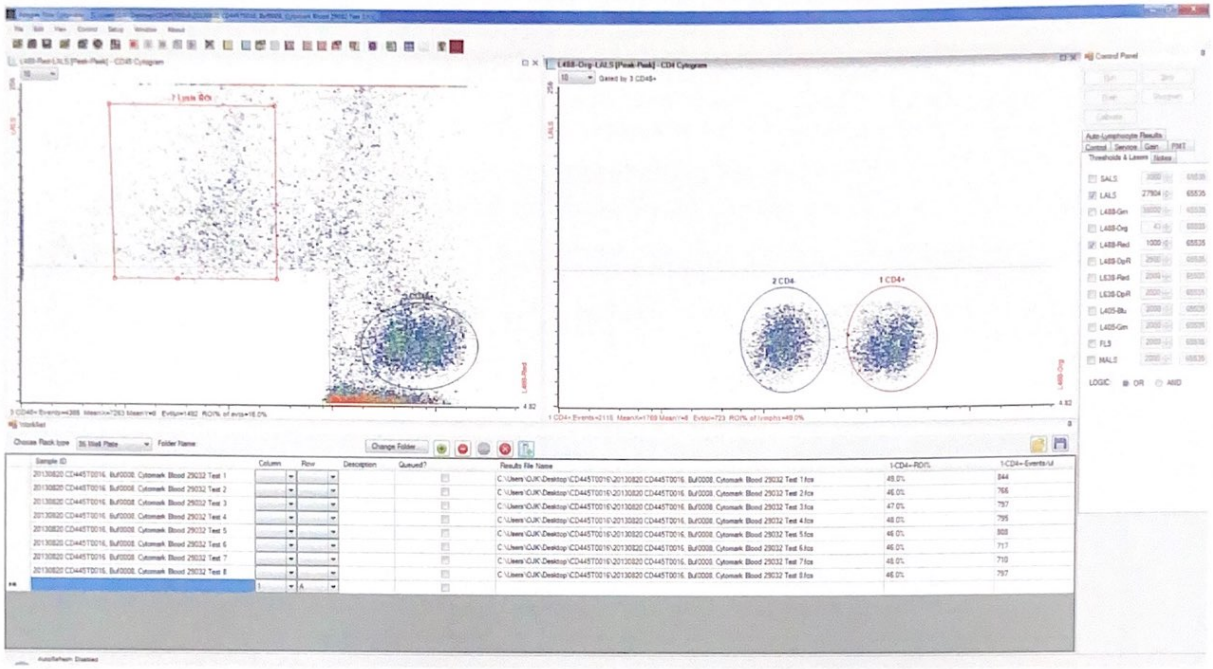


Work List
Samples must be given an ID, a location and must be Queued in order for them to be processed by the Autosampler. The Work List can also be used to compile a comparison of previously saved datafiles by adding columns for ROI statistics and then loading each datafile in turn.

The Work List can also be used to compare data from multiple samples. This is done by:

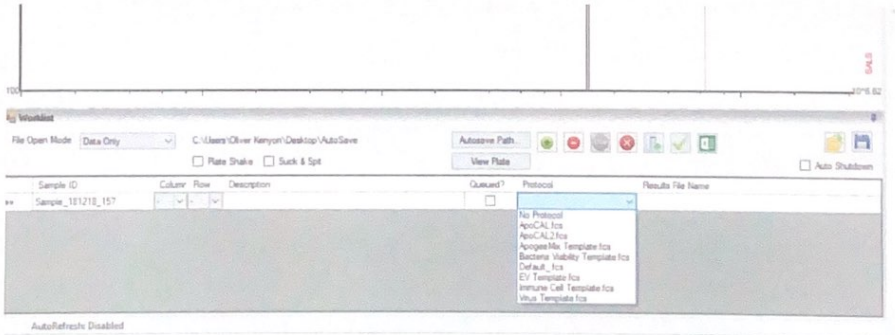
1) Creating appropriate ROIs
2) Using the 'Add ROI Statistic' button to add columns to the WorkList to display the statistics of interest
3) Using the 'Load Data Only' option on the File Menu to get data from a previously run sample
4) Clicking the Work List button 'Add Datafile to WorkList' to add the current file's information to the Work List.

An example is shown below.



Refer to the AutoSampler Chapter for more information.

Histogram application version 255.0.0.205 and later offers the ability to specify a settings template to use for each sample. The template options are the files stored in the folder C:\Program Files (x86)\Apogee\Protocols



7.7 Axes Calibration (Optional)

The optional calibration module allow a calibrated scale to be displayed on any axis. The scale must be configured in the factory or during an instrument service visit. Calibration scales may be plotted along an axis (for fluorescence) or in two dimensions on a light scatter cytogram as shown below. Available scales may be selected for display or hidden via the Datagram Context menu (right clicking on the datagram).

The below graph shows the display of calibrated scales on a two dimensional light scatter 'cytogram'. These curves are for η=1.42 and η=1.38, but other curves can be added and a gate or ROI defined according to the size and refractive index of interest.

Apogee's flow cytometers can be calibrated to give size information from the light scatter data for particles of approximately known refractive index. Bacteria and similar particles generally have a refractive index of around 1.38 but conventional flow cytometers are calibrated using only latex beads with a refractive index of 1.59. Due to this large refractive index difference, latex beads can give misleading results if used to infer biological particle size by their light scatter signals.

Apogee's Light Scatter Calibration Module (Cat# 1492) offers a scale, calibrated for a particular refractive index, on the light scatter histograms. The instrument is set up during an instrument service visit or in the factory. Calibration can be done at almost any refractive index greater than the refractive index of water, and multiple scales can be displayed on any light scatter cytogram to allow the operator to capture particles within a particular size and refractive index range.

The calibration scales can also be used to display fluorescence calibration data so that the number of Molecules of Equivalent Soluble Fluorochrome (MESF) can be determined.

For further information refer to Apogee document #16023 for product #1492

8. Routine Operation

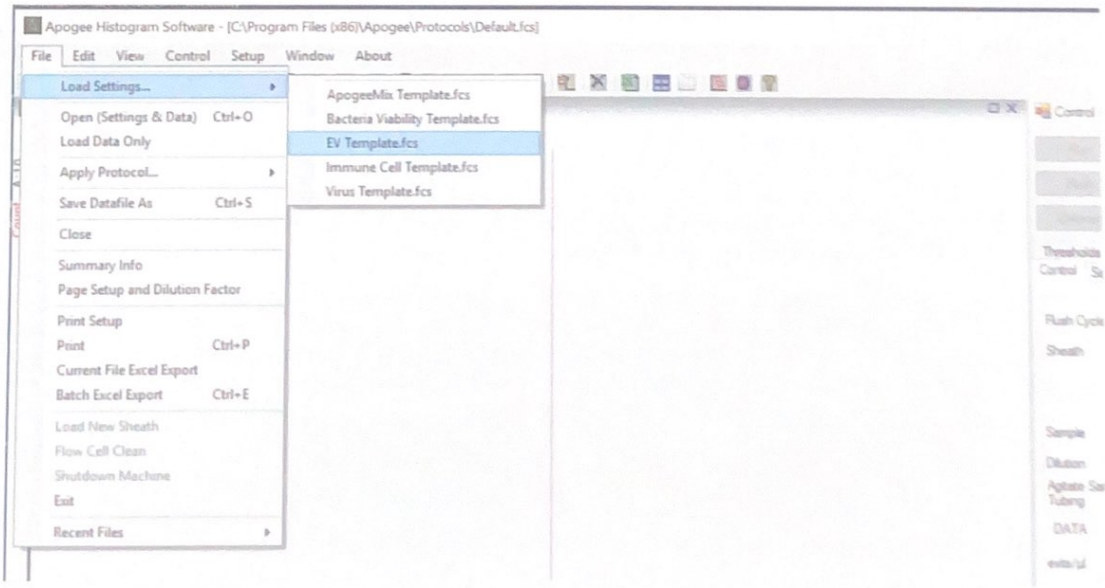
It is generally recommended to verify the cytometer's performance with reference particles (test beads) at the beginning of each session of use. The demands of the application will determine how thoroughly it is necessary to check the instrument's condition. For example, use in a GMP environment may require a thorough daily control including analysis of the following samples at the beginning and end of each session of use:

- Running a mixture of test beads (e.g. ApogeeMix, Cat#1493) to verify the machine has the normal light scatter performance and the normal fluorescence performance on green and orange detectors on blue and violet lasers.
- Running other test beads to verify the cytometer has the normal fluorescence performance on any other fluorescence detectors (e.g. Cat# 1478 for detectors on the red laser, Cat# 1444 to take red fluorescence measurements from fluorescence detectors on the blue and violet lasers.
- Running a sample of purified water with a low light scatter trigger threshold to verify the fluid systems are clean. For example, for Micro models set a light scatter trigger threshold to the level where 110nm polystyrene beads appear; for Universal models set the light scatter threshold to where 200nm polystyrene test beads appear. A clean cytometer should then give fewer than 200 events per second when running a sample of purified water at 3ul/min.
- Running beads of carefully controlled concentration (e.g. Apogee Cat#1444) multiple times to verify the counting precision and accuracy.

Templates for the test bead samples and also for common biological samples may be stored in

C:\Program Files (x86)\Apogee\Protocols

so they appear on the *File---Load Settings* pull-down menu (see image below).



8.1 Operation with the "Histogram" application

8.1.1 Instruments without ApoCAL calibration option (non-GxP models)

8.1.1.1 Start-up & QC

1) If using a separate PC for the histogram software, turn it on now. Windows must have started on the external PC before you turn on the cytometer in order for the cytometer to know the external PC is connected. See section 6.3, Network Configuration for how to set up the LAN connection.
2) If the cytometer has the manual sample arm (no Autosampler) check the sample arm is in the flush position.
3) Fill the sheath fluid tank with deionized water containing 200µl of Sheath Fluid Preservative (Apogee Cat#1516) per full tank of water. Empty the waste tank. Top up the cleaning fluid tank if necessary.
4) Turn on the cytometer using the push button on the front panel.
5) Windows is normally set to automatically run the FCM Control program. Once the machine is Initializing (not before), the FCM Control program is normally set to automatically run the histogram software. The firmware version should be momentarily displayed, indicating a connection has been established between the histogram software and the cytometer. If the connection fails make sure only one copy of the Histogram software is running (check Windows Task Manager for instances which may have failed to close properly) and refer to sections 6.3 and 7.1.
6) After the Initialize cleaning cycles are complete, the instrument status bar in the histogram software will say "STATUS: Idle Clean".
7) Load your reference test bead settings file.
8) Run a sample of your reference test beads and check that the data is sufficiently similar to your standards. For example verify the CVs and signal strengths are within acceptable limits.
9) Daily or weekly, depending on how critical the counting accuracy is in your application, check the counting accuracy as follows:
   i) Run some Apogee calibration beads at about 1.5ul/min for approximately 200 seconds and record the concentration of particles (evt/ul) in the singlet peak.
   ii) Run the same beads again at 10ul/min for 100 seconds.
   iii) Verify that the measured particle concentrations agree with one another to within 15% and agree with the value on the bottle to within 15%.
10) Run a sample of purified water or buffer to check the fluidic cleanliness. For Micro models, set a trigger threshold at the position of the 110nm polystyrene test beads on the MALS or LALS detector and make sure there are fewer than 250 events per second. The event rate typically falls during the first 20 minutes of operation.

Note that when the histogram software is run, the file:

C:/Program Files(x86)/Apogee/Protocols/Default.fcs

is automatically loaded. This file contains stored data and does not indicate the current state of the machine. This file can be modified to your reference calibration settings.

During installation of the machine, suitable settings files and reference data should be discussed.

8.1.1.2 Running a Sample

If the controls in the previous section are successful, the instrument is ready to analyze biological samples.

1. Click the  icon on the toolbar; In the Open dialog box, locate the desired data file and click on the Open button. Alternatively load settings from the File---Load Settings menu option.
2. Make sure a file AutoSave path has been entered in the WorkList header and enter a sample ID for the sample you are about to run into the work list.
3. To run the sample which should have a tick in the Queued column of the Work List, move the sample arm to the Run position or simply click the Run button on Autosampler models.
4. When the instrument starts collecting data, move the sample arm to the flush position (manual sample models) so the machine can flush automatically when the acquisition stops.
5. After each sample, the datafile will be automatically saved with the SampleID as file name.

Sample load/run position

Intermediary position when moving between 'run' and 'flush'

'Flush' position

If an Autosampler is installed then a 'Manual Sample' can be run simply by entering the SampleID in the Work List, inserting a tube in the transparent acrylic tube holder and clicking the 'Run' button on the Control Panel. See section 9 for further details.

8.1.2 Instruments with ApoCAL calibration option enabled (GxP option)

8.1.2.1 Start up & Auto-Calibration

The software may be configured to operate in 'GxP' mode (refer to section 7.1.3), suitable for use in regulated environments where data integrity and ease of use are high priorities. The system automatically calibrates to ApoCAL test beads (Cat#1524) and user permissions are limited to minimize the chance of false results.

The ApoCAL software (included in Cat# 8057) algorithm operates in the FCM Control program. The Histogram application does not participate in the calibration algorithm other than to display instrument status information, to turn Calibration mode on and off and to set instrument settings suitable for the calibration which it obtains from the file C:\Program Files (x86)\Apogee PC Control\ApoCAL.fcs

During the self-calibration, the FCM Control program adjusts the PMT voltages and checks the counting

accuracy. File C:\Program Files (x86)\Protocols\'ApoCAL.fcs contains suitable settings for the ApoCAL test bead mixture (Cat#1524). This protocol/template will be loaded each time the machine performs a calibration.
To instruct the machine to perform a calibration, queue a sample (in the WorkList) with Sample ID beginning with the characters "ApoCAL" or click the Calibrate button (level 3 users only) on the Control Panel of the Histogram application.

The following steps should be followed to turn on and calibrate the machine each session:

1) Fill the sheath tank. Check the Cleaning fluid tank is at least one third full. Empty the waste tank.

2) Switch on the machine. The machine may start to clean itself before a user has logged in.

3) Log in to Windows.

4) After one or two minutes Windows will automatically run the Histogram application and a default file will load.

5) While waiting for the machine to finish its "Initialize" cleaning routine (approx. 5 mins), click the AutoSave path button in the WorkList header and set a new folder for this session's data.

6) Insert a microcentrifuge tube containing approx. 0.5ml of ApoCAL beads (#1524).

7) Wait for the initialize routine to finish and check the machine's status is "Ready for a Sample". The instrument status bar in the histogram software should say "UNCALIBRATED: Idle Clean".

8) Enter the SampleID "ApoCAL" in the WorkList then press F10 or click the Run button. For manual sampling machines move the sample arm to the Run position.

Allow the machine to analyze the sample. To position the test beads in the correct position it may automatically adjust the PMT voltages on up to three occasions, each time clearing the data buffer. Allow the machine to analyse the sample for at least 90 seconds.

The instrument calibrates itself by positioning the calibration bead populations on the histograms and by checking the number of particles per volume of sample. If the concentration of the measured calibration beads is outside the normal, or if the brightness of the measured calibration beads differs from the expected brightness by more than is normal, the machine will tell you "Calibration Failed".

The calibration status is displayed on the Control Panel and in the status bar. Do not operate the machine unless it is in the Calibrated state. A log file of calibration results is maintained in:

`C:\Users\Public\Documents\Apogee`

8.1.2.2 Dilution Factor

A 'Dilution Factor' may be set in the histogram software so that the machine calculates the correct particle concentration from samples which have been diluted. For example, white blood cells are often analysed in whole lysed blood at 20ul/min after dilution by a factor of 10; blood plasma is often diluted by 100x before analyzing its content of extracellular vesicles at 3ul/min.

If you run undiluted test beads with a dilution factor set, the histogram software reports a 'corrected' concentration to allow for the dilution. So, for example, on the bottle it may say that the beads are 1,000 events per microlitre, but if a 10x dilution factor is set, the histogram software will report 10,000 per microlitre. The dilution factor setting has no effect on the FCM Control program in which the calibration is

performed, so dilution of the ApoCAL test beads will cause the calibration to fail regardless of the dilution factor.

8.1.2.3 Running Samples

After a successful calibration, the instrument is ready to analyze your samples. The calibration state is also displayed on the status bar and Control Panel in the Histogram application.

An AutoCycle should have been set in the protocol/template files for the ApoCAL samples and for the biological samples. These settings automatically stop the sample after a set period of time and/or when a certain number of particles have been detected.
The AutoSave option in the WorkList header should be set and then SampleIDs entered in the WorkList for the set of samples you intend to run. The data will then be automatically saved with the SampleID as the file name when each sample stops.

8.2 Shut-down procedure

To clean and disinfect the system after running biological samples it is recommended to shut down the machine as follows:

1. Run a 200µl sample of 1% sodium hypochlorite (10% bleach) solution at 50µl/min until the sample runs out.
2. Set a relatively low light scatter trigger threshold. The trigger level should be chosen to exclude optical noise but low enough to allow triggering on particles in the sample and sheath system. For Micro and Micro-PLUS models you may set the light scatter trigger threshold to the level of 110nm polystyrene beads. For Universal models you may set the light scatter trigger level approximately 1 decade below the level of 500nm polystyrene test beads.
3. Run a sample of filtered water at about 5µl/min and judge the cleanliness of the system by the event rate. A clean instrument should give less than 250 events per second (trigger level dependent).
4. Repeat the water sample until the event rate is sufficiently low to indicate the machine is clean. Failure to ensure the machine is clean in this way risks damage to the flow cell due to deposits of proteins on the optical surfaces of the flow cell crystal.
5. Click the “Shutdown” button on the Histogram Software Control Panel and allow the machine to perform its cleaning cycles and turn off.

Never switch off the instrument without performing the shutdown procedure because the fluidics may be left in an unclean state

9. AutoSampler Operation

The optional AutoSampler is a convenient system to process large numbers of samples. It requires racks which conform to the industrial standard 96 well plate format.

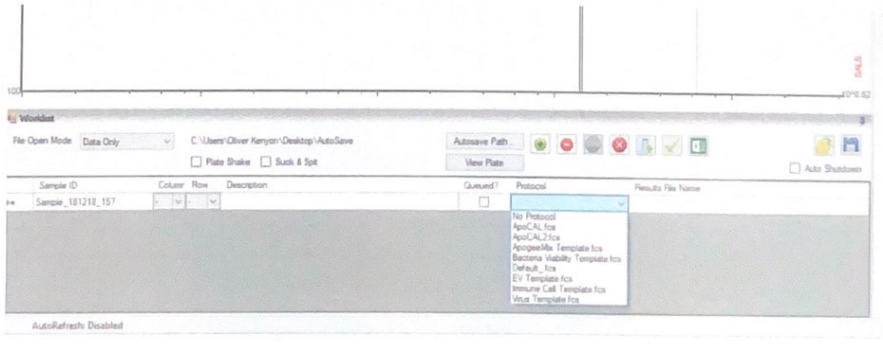
Control of the AutoSampler is normally via the Work List panel in the Histogram Software, but 'Manual' samples may be run by placing a sample tube in the Manual sample position (see photo). A sensor detects the presence of a sample in the manual sample position and the manual sample will be given preference over the rack of samples.

In between each sample, the sample aspiration probe automatically moves into the Flush Cup for cleaning.

If no sample is present in the Manual sample position, the computer will process Queued samples defined in the Work List and save each datafile in the assigned folder. Each sample must be given an ID, and must have its 'Queued' checkbox ticked.

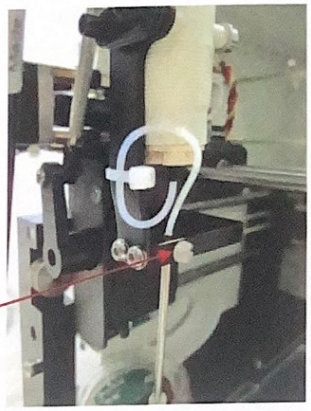
ROI statistic columns can be added to the Work List table to facilitate comparisons between samples.

Histogram Software version 255.0.0.205 and later offers the ability to specify a settings template to use for each sample. The template options are the files stored in the folder C:\Program Files (x86)\Apogee\Protocols



9.1 AutoSampler Probe Height Adjustment

The probe height must be considered when deciding on the volume of sample to use in the microcentrifuge tube or plate well. If the probe does not reach the bottom of the container then, of course, more sample is required in the tube to avoid aspiration of air. The probe height may be adjusted by loosening the nylon clamp screw.



9.2 AutoSampler Fault Diagnosis

When the instrument first turns on, during the Initialize procedure, a test of the mechanism is performed to make sure it is free to move throughout its full range. Apogee's Terminal program can be used by Advanced users to check whether this 'Self Test' passes or fails.

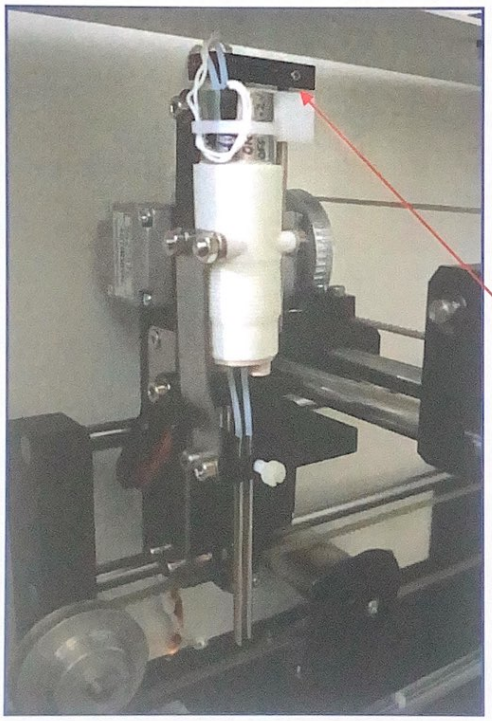
The stepper motors which drive the mechanism in the horizontal plane, have limited power to minimize the risk of damage in the event of a failure or obstruction. The shafts require periodic cleaning and lubrication with grease to ensure there is no significant resistance to movement.

The AutoSampler electronics is interfaced with the PC via USB connections. If these communications have failed, or if the 'Z' sensor has been interrupted, it may be necessary to restart the instrument. After a re-start, if there is still no movement of the AutoSampler during the Initialize procedure, and if all the sensors have been checked, it is possible that the driver for the USB communications has become failed. In extreme cases it may be necessary to unplug the USB cables for the AutoSampler, restart the PC, then plug in the USB cables again. Consult Apogee before doing this as the cables should be connected in the correct order (although the order can be changed in the FCM Control program).

The AutoSampler is fitted with the following sensors:

SENSOR	DESCRIPTION	NOTES
Cover sensor	Micro switch to detect a closed cover	Mechanism will not move unless the cover is closed. Software will wait for approx 15 seconds for the operator to close the cover and LEDs may flash to indicate this error state. After this period the user will need to click 'Run' or 'Flush' in the software after closing the cover.
Manual Sample Sensor	A small sensor to detect the presence of a tube in the 'Manual Sample' position	Manual samples have priority over samples queued in the rack of samples
X motion sensor	Detects when the mechanism reaches the end of travel in the X direction (longest	Self test at startup verifies that the mechanism is free to move the full distance (use of Terminal program is required to verify)

	movement)	
Y motion Sensor	Detects when the mechanism reaches the end of travel in the Y direction (shortest movement)	Self test at startup verifies that the mechanism is free to move the full distance (use of Terminal program is required to verify)
Z motion sensor	Detects when the sample probe is prevented from lowering into a sample or into the flush cup.	This error is treated seriously and the instrument will need to be restarted to recover from this state. Until a restart, the Autosampler will not function.



The 'Z' sensor detects a gap between these two blocks. This fault condition can happen when the aspiration tube(s) hit an obstruction during the downward movement (for example due to a misaligned rack of samples). The sensor is located on a small PCB fi9xed to the sliding block on later models.

10. Troubleshooting: Common questions

The data looks abnormal but the instrument settings are thought to be correct.
The histogram software doesn't communicate (cannot connect) with the FCM Control software.
The sample does not automatically run when the sample arm is moved to the run position.
The flush doesn't start automatically.
The acquisition rate (events/second) is erratic.
The background light level is high (this should be less than 1.0 for all detectors)
Incorrect calibration bead histogram channel number: the calibration beads fail to appear in the correct histogram channel (+/-20%) with standard settings.
The absolute counting accuracy of the machine is poor. When Apogee calibration beads are run at 2.1µl/min for approximately 200 seconds, the concentration of particles (evt/µl) in the singlet peak is more than 15% different from the labeled value.
Poor CV from test beads
When running a purified water control sample, too many events are counted.

See the below table for answers to these common questions.

What happens	Why	To do
The data looks abnormal but the instrument settings are thought to be correct.	Dirty tubes or flow cell. Reagent problems.	Run test beads followed by a sample of filtered water or buffer to verify the fluid tubes are clean. A 'Flow Cell Clean' operation may help to reduce optical noise but will not reduce dirt particles in the fluid system.
The Histogram Software doesn't communicate with (cannot connect to) the FCM Control software.	If running the two programs on the same computer inside the machine: • If the LAN connects after the FCM Control software has started, it will disrupt the connection between the programs. The LAN connection must not change after the FCM Control program has been run. Some network security programs may not be compatible with the Apogee software or may require than the FCM Control program be started manually (instead of from the Windows Startup folder), after the LAN is fully established. • Multiple copies of software running (check the Windows Task Manager) due to operator error or due to a crash which prevented the program from closing properly. • Incompatible LAN connected (disconnect from LAN and restart to verify) • TCP/IP LAN configuration error	Reboot the machine and the PC. Check the network cable connection on the rear panel of the cytometer and PC. Repeat the instrument start up procedure (above). Check that the 'Connect to FCM' check box is checked in the User Configuration program. Check the correct network name or IP address is specified in the User Configuration program.
The sample does not automatically run when the sample arm is moved to the run position.	The software polls the sensor for a change in the sample arm position. Sometimes the timing may miss the position change. Repeat the movement or press the 'Run' button on the Control tab. If the sample still does not run then check that the test tube is suitable (too short or too long); it must not prevent the sample arm from moving fully up into position.	Transfer the sample to an appropriate test tube. The sample needle probe is clamped by a nylon screw. The height of the sample needle can be adjusted to allow use of your preferred sample tubes.
The flush doesn't start automatically	Try clicking the 'Flush' button (green button) on the FCM Control program. If this does not fix the problem then the sample arm is not fully in the flush position.	Move the sample arm to the flush position

What happens	Why	To do
The acquisition rate (events/second) is erratic	There may be an air bubble in the sample syringe or the sample syringe may be worn. The sheath filter may be partially blocked due to a large amount of air in its inlet.	Click the 'Remove Air in Syringe' button on the Service tab of the Control Panel. This automatically removes air from the sample syringe. Refer to section 11.2 and then 11.4 Refer to section 11.5 to test the sheath fluid filter.
The background light level is high: It should be less than 1.0 for all detectors)	The flow cell is dirty or the PMT voltage is too high	Run a flow cell clean routine from the Service tab of the Control Panel. Perform a flow cell cleaning fluid soak (button on Service tab) As a temporary work around, reduce the PMT voltage and use software Gain if necessary to move the populations back to the normal position.
The background light level is high (> 1.0)	The OL&FC assembly (flow cell) is damaged	Contact Apogee for technical assistance. (support@ApogeeFlow.com)
Incorrect calibration bead histogram channel number: the calibration beads fail to appear in the correct histogram channel (+/- 20%) with your standard settings (PMT Voltages, Gain Settings, Subtraction settings))	Optical fault	Check the background levels (see above) and if necessary perform a flow cell clean procedure. If the background levels remain high, use more software gain and lower PMT voltages.
Incorrect calibration bead histogram channel number	Fluidics fault	Check the connections to the fluid box. Perform a 'New Sheath' and 'Flow Cell Clean' procedure (service tab of histogram software) to remove air from the sheath fluid system and clean the tubes. Perform the 'Remove Air in Syringe' procedure from the Service tab of the control panel.

What happens	Why	To do
The counting accuracy of the machine is poor. When Apogee calibration beads are run at 2.1µl/min for approximately 200 seconds, the concentration of particles (evt/ul) in the singlet peak is more than 15% different from the labeled value.	The test beads have deteriorated (e.g. clumped into more doublets, triplets etc.) OR: Air may have collected in the sample syringe. OR: The syringe is damaged or worn.	Click the 'Remove Air in Syringe' button on the Service tab of the Control Panel. This automatically removes air from the sample syringe. Refer to section 11.2 and then 11.4
Bad CV on calibration sample	Remember that high sample flow rates give high CVs. An air bubble stuck in the flow cell may be misaligning the sample stream in the laser.	Check that you have selected the normal sample flow rate. Click the 'Flow Cell Clean' button on the Service tab to dislodge any air bubbles.
	Low sheath flow rate gives high CVs	Check the sheath pressure (sheath fluid flow rate) is set normally. Try increasing it.
When running a purified water control sample, too many events are counted.	Events are triggered by detectors with an active trigger threshold with. The cause may be: 1. Optical noise spikes above the trigger level; the PMT noise level is high, OR 2. A dirt particle in the sheath fluid passes through the laser; the sheath fluid system is dirty, OR 3. A dirt particle in the sample or released by the sample system tubing passes through the laser; the sample system is dirty	Check the PMT noise levels on the PMT tab of the histogram software. Change a voltage by 1V to make the noise values update after 10 seconds. If the noise is elevated (it should always be kept less than 1.00), perform Flow Cell Clean cycles and Flow Cell Soak cycles to reduce the optical noise. Establish whether the source of dirt particles is the sheath fluid system or sample fluid system by observing the effect of changing the sample flow rate. If the source is the sample system (or sample), the event rate will be roughly proportional to the sample flow rate. If the source is the sample system, run a cleaning fluid as a sample and perform several Flush cycles. If the source is the sheath fluid system, run a long sample of water and monitor the event rate over 30 minutes to see whether it cleans up. If not, the sheath fluid filter and tubing may need to be replaced.

11. Service and Maintenance Procedures

11.1 Routine Maintenance

Apogee Flow Cytometers have fully automated cleaning routines so that no operator maintenance is required under normal operating conditions. The user should, however, regularly run calibration beads to monitor the general condition of the machine by checking:

- counting accuracy and precision
- for changes in the fluorescence and light scatter signal strengths and CVs
- light scatter noise level (the amount of background scattered light in the flow cell, see PMT tab of histogram software)

To clean and disinfect the system after running biological samples, be sure to follow the Shutdown procedure provided in section 8.4 to make sure the system is clean before it is turned off.

The recommended Preventative Maintenance interval ranges from 6 to 18 months depending on the operator skill level and the application. Apogee offers a performance assessment service via remote desktop connection which can help to ensure service visits are scheduled efficiently.

For maintenance procedures requiring removal of the covers, it is recommended to first turn all lasers off using the check boxes on the Control Tab of Histogram Software. If the covers are removed and laser(s) are turned on, the machine is then a Class 2M laser product; the system is safe for accidental exposure providing optical instruments are not used to view or divert the laser light.

11.2 Testing the Sample System and Replacing the Syringe

The syringe should be replaced annually or whenever the system fails the tests described in the following section.

11.2.1 Testing the sample system

The most common sample system faults are a worn syringe or air in the syringe. They can seriously affect counting accuracy and precision.

If the counting accuracy is poor, automatically remove any air from the syringe by clicking the button on the Service tab of the histogram software's Control Panel.

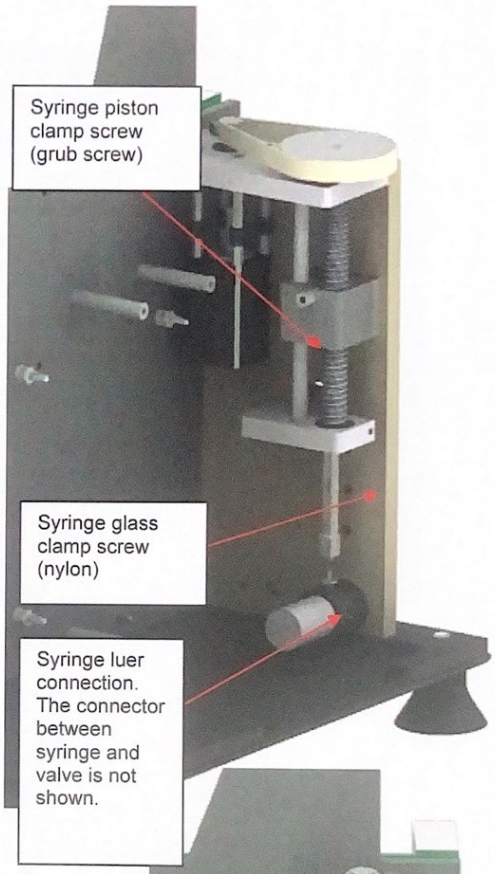
Counting Accuracy Test:
To check for a worn syringe (or air in the syringe, or a leak or blockage in the sample fluidics) is to measure the events per microliter at several sample flow rates. For example compare the events/µl at 1.5µl/min to 10.5µl/min, remembering to allow the event rate to stabilize after changes in the sample flow rate.

Evts/µl values consistent to within 10% should be obtained for flow rates in the range 1.5 to 10.5µl/min. The total counting rate (total event rate) should be kept below 10,000 per second to ensure the electronics is not limiting the count rate. On older models (A20 and A40 generations) keep the event rate below 2,000 per second. If necessary dilute the sample.

If the counting precision remains poor after changing the syringe, test the syringe valve as explained in section 11.4.

To remove the syringe:

- Shut the machine down
- Remove the right hand side cover
- Accurately record the position of the syringe piston in the glass syringe. The height of the syringe piston is critical to ensure the 'Remove Air in Syringe' routine functions properly. Also note the length of piston protruding above the grub screw clamp bar.
- Loosen the plastic screw which clamps the glass syringe.
- Loosen the small grub screw which clamps the syringe piston (1.5mm 'across flats' (A/F) Allen key for old type syringe, 2mm A/F for new type syringe)
- Gently turn the syringe pulley wheel to move the syringe arm upwards to allow you to remove the syringe more easily.
- Grip the plastic connector which joins the syringe to the valve in order to stop it from turning when you unscrew the syringe's Luer connector. With your other hand, grip the flange at the top of the glass syringe and unscrew the syringe from the plastic connector between the syringe valve and syringe.
- The syringe can then be removed. The Luer connector only requires about 1 turn of the syringe.

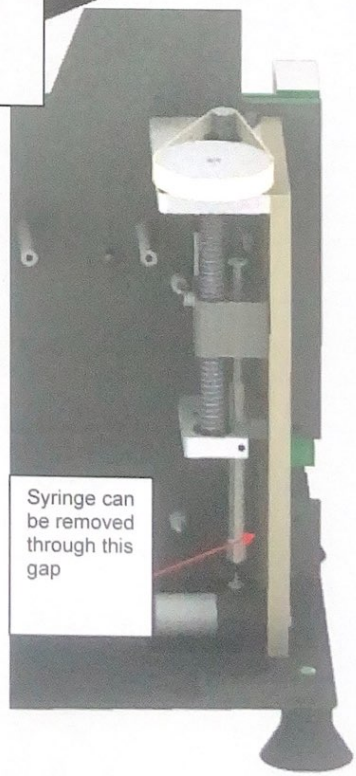


11.2.2 Mounting a new syringe

Syringe replacement is essentially the reverse of the removal procedure.

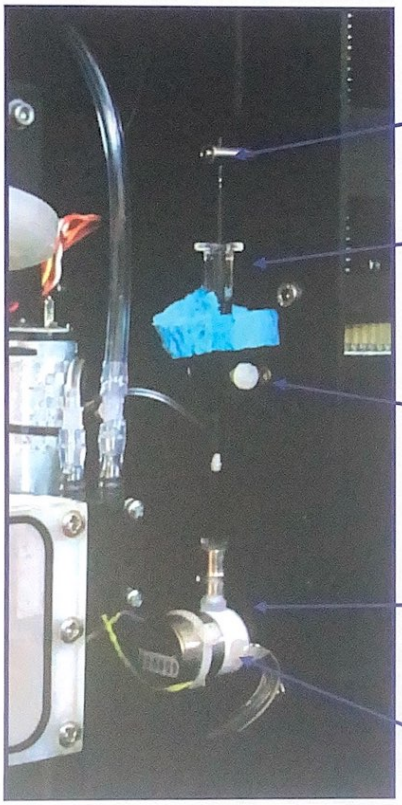
However, it is very important to make sure the syringe is clamped at the correct height to ensure the 'Remove Air in Syringe' routine works properly. The syringe glass is tapered for a distance of approximately 4mm from its opening. During the 'Remove Air in Syringe' routine, the syringe motor drives the piston to this tapered section to completely fill the syringe and to allow any air bubbles to escape by gravity. The syringe piston height must be set precisely for this to operate correctly. The piston must reach the tapered section of the glass so that any air bubbles can escape, but the piston should not escape completely from the glass (1mm should remain inside) to avoid the possibility of the piston not returning correctly into the glass.

Furthermore, the length of the syringe glass is not always consistent from syringe to syringe, so after fitting a new syringe it is essential to verify the piston is set correctly by performing a 'Remove Air in Syringe' operation, and checking that all but 1mm of the piston comes out of the glass, and checking that a little water flows out of the syringe (taking any air bubbles with it).



After replacing the syringe, air must be removed from the new syringe. This is most easily done by performing several 'Remove Air In Syringe' operations by clicking the button on the Service tab of the Control Panel in the Histogram Software. Watch the mechanism as you do these to ensure all air is indeed removed from the syringe.

A40 generation cytometers are fitted with a different type of syringe drive, but the principles are the same. See image below.



Old Type Syringe Drive

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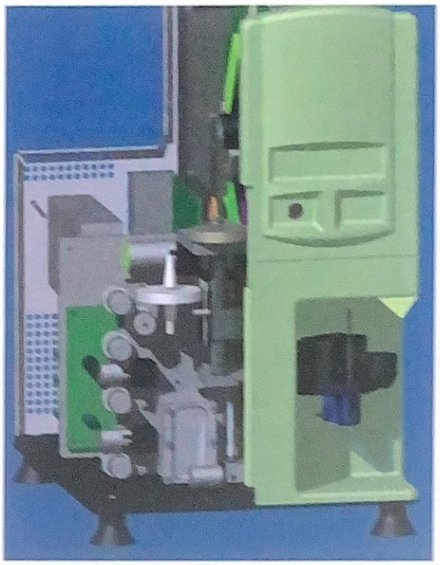
A60 generation cytometers have the syringe drive mounted at the front of the instrument for easier access when an Autosampler is fitted, but the procedure for changing the syringe remains the same as for the A50 generation.

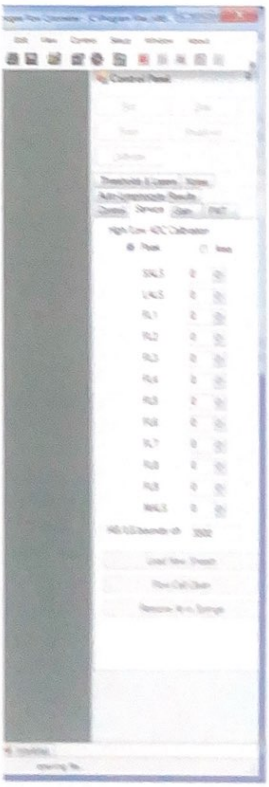


Notes:

After removing the syringe, a worn syringe can be sensed by gently pumping the piston in and out and feeling whether the resistance is uniform along the length of the syringe, and comparing the resistance to piston movement with a new syringe.

The 'Remove Air In Syringe' button is disabled unless the machine is idle and ready for a sample. The sample arm must be in the flush position in preparation for the flush cycle which occurs after the air has been removed.

Note that air is removed by gravity (the weight of water in the sheath tank). If air gets sucked into the syringe instead of water flowing out of the syringe, then there is a problem with the sheath fluid filter or the supply of sheath fluid from the tank. See Advanced User Manual section named "Fluid Section: Technical Description" for details.



11.3 Flow Cell soak in cleaning fluid to correct elevated light scatter PMT noise levels

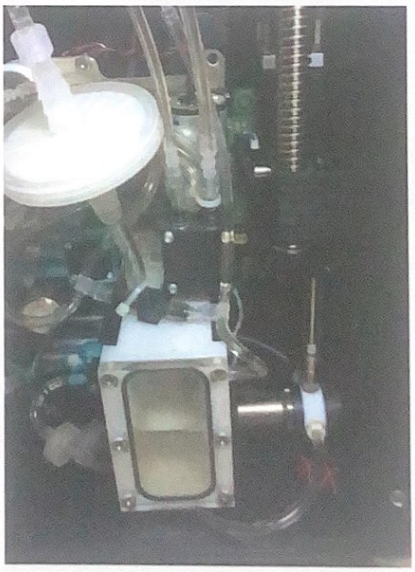
Over time, the light scatter PMT noise levels may increase due to deposits on the optical surfaces in the flow cell. To correct this is may be necessary to soak the flow cell in cleaning fluid for longer than is done during the Flow Cell Clean routine. Histogram software versions after 255.0.0.155 offer a "Flow Cell Soak" button on the Service tab.

If an earlier version of software is installed, it is possible to clean these surfaces by manually controlling valves and pumps using the Terminal program installed on the cytometer's computer. The procedure is as follows:

a. With the machine idle and all lasers turned off
b. Run the Terminal program
c. On the "Vac Pmp" tab click "Vacuum Pump Speed 3"
d. On the "Valves" tab click "Clean Valve"
e. Wait 30 seconds
f. On the "Vac Pmp" tab click "Vacuum Pump Off"
g. On the "Valves" tab click "Turn Off Valves"
h. Leave the machine for 20 mins for the flow cell to soak with cleaning fluid inside
i. Tell the machine to Flush three times (button on Control Panel of the Histogram Software)
j. Run a sample of water.
k. Turn the laser(s) back on and change any of the PMT Voltages by 1 Volt to cause the machine to read the noise levels
l. If the noise levels are significantly lower you may repeat this process until the noise levels are normal.

11.4 Syringe valve test (event rate instability/poor counting precision)

To address poor counting precision, first consider the steps in section 11.2: worn syringe or air bubble in the syringe. If the events per microliter readings remain imprecise after changing the syringe, consider a possible faulty syringe valve.



If the syringe valve does not seal correctly, the counting precision will be poor and the events per microliter will be erratic. Typically the events per microliter will be elevated at very low flow rates and suppressed at high sample flow rates.

The syringe valve connects the syringe to a supply of sheath fluid when turned on, or to the flow cell (via the sample valve) when turned off.

The syringe valve may be tested while running a sample by monitoring the event rate (plot a histogram with TIME on the x axis) when you squeeze the sheath supply tube (see red crosses in photo) and hold it closed for about 10 seconds. Squeezing the syringe valve's sheath fluid supply tube should have no effect on the event rate. If the valve is failing to seal, the event rate will change when you squeeze the sheath supply tube and the valve should be replaced.

11.5 Testing and re-wetting the sheath fluid filter

If the sheath fluid filter cartridge is larger than the cleaning fluid filter cartridge, it will be a hydrophobic type which blocks water flow if the cartridge fills with air. If the sheath tank has been allowed to run dry, air will have been sucked into the sheath filter and the membrane may require re-wetting with alcohol (ethanol or methanol). The test and rewetting procedure is as follows:

Sheath filter test:

1. Turn off the machine
2. Remove the fluid tank
3. Remove the cytometer's side cover on which the fluid tank normally sits.
4. Disconnect the luer connector on the Sheath fluid filter outlet tube and place it in a large beaker.
5. Secure the side cover so it cannot topple over while still allowing you access to the sheath filter outlet tube.
6. Top up the sheath tank so that the height difference between the water in the sheath tank and the end of the tube in the beaker is about 30cm.
7. Re-connect the fluid tank
8. Water will now flow from the tank, through the filter, into the beaker. Measure the flow rate; it should be at least 45ml per minute.

If the flow rate under 30cm of gravity is too low, the filter membrane should be re-wetted with alcohol.

Filter wetting procedure:

1. Pull off the blue connector (the connector which normally connects to the sheath tank) from the tube on the sheath filter inlet.
2. Water will now flow by gravity into the beaker, filling the filter inlet with air. If the filter inlet does not fill with air, tilt the filter unit and try to empty water in the inlet side of the filter out through the filter cartridge bleed port (disconnect the Luer connector). It is important to eliminate most of the water from the unfiltered (inlet) side of the filter cartridge.
3. When the inlet side of the filter cartridge is full of air, plug the filter outlet tube (the tube in the beaker).
4. If not already done in step 10, disconnect the filter bleed port tube (Luer connector) to allow air out of the filter in the next step when you inject alcohol.
5. Inject approximately 100ml of ethanol (>95%) into the filter until it starts to flow out of the bleed port.
6. Reconnect the tube to the filter bleed port Luer connector.
7. Reconnect the blue connector to the sheath fluid inlet tube.
8. Connect the blue connector to the sheath tank and unplug the filter outlet tube so water flows into the beaker.
9. Measure the water flow rate. It should be at least 45ml/min with 30cm height of water (tank water level to beaker).
10. Allow at least 2 litres of water to flow through the filter to flush away the ethanol.
11. Disconnect the sheath tank and remove it from the cytometer's side cover.
12. Reconnect the sheath filter outlet tube's Luer connector.
13. Replace the cytometer's side cover.
14. Reconnect the sheath tank and test that the problem is solved.

11.6 Packing the Cytometer for Shipment

If the cytometer needs to be transported, it is best packed on a pallet following the below recommendations.

To pack an ApogeeFlow cytometer

- Empty all fluid tanks
- Ship the cytometer upright. It is not designed for shipping in any other orientation
- Preferably put inside an ApogeeFlow travel case as shown in the images below
- Always ship on a pallet because the pallet gives protection against big impacts and ensures the system is kept upright
- Use a generous layer of firm foam between the cytometer and the pallet
- Strap the cytometer to the pallet with strong strapping

When sending a cytometer to ApogeeFlow for repair

- Decontaminate the system before shipping by wiping surfaces with a disinfectant
- Do not send the waste tank, monitor, keyboard, mouse