Dako

CyAn™ Field Service Manual

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Scope

This manual is written for Dako Field Service Representatives only. This document is for internal company use only.

Disclaimers

This document is not a substitute for the detailed service training provided by Dako, or for other advanced instruction in general cytometric techniques.

Trademarks

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Contents Overview

This service manual contains information for Dako Field Service Representatives. Topics include: CyAn Safety, Instrument Overview and Alignment, Summit Data Acquisition and Analysis Software, Installation and Lab Prep, Technical Specifications, Customer Service and Sales, Bench Layout and Integration, Troubleshooting, CyAn Applications, and Repair and Replacement. An appendix of reference materials is also included.

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Section 1

Safety

The CyAn product line has been engineered with safety as one of its primary features. Safety of the operator, of bystanders, and of valuable samples is paramount to Dako's commitment to high performance design and engineering. The three distinct types of safety hazard concerns are Electrical Shock, Laser Irradiation, and Biohazards.

Electrical Safety
Symptoms of Electrical Malfunctions
During normal CyAn ADP operation you should watch for the smell of hot electronics, excessive heat, or electrical arcing. If any of these occur, immediately power off the CyAn and determine the location of the malfunction. Also check for any fluidic leaks that may have caused sheath to fall on to the electronics. Replace all defective parts as needed.

Laser Safety
The CyAn product line conforms to international regulations encompassing laser safety. Most laser products used as components of the CyAn Analyzer are Class IIIB to Class IV. As such, these lasers have the potential to cause injury. **The CyAn is a Class I laser device**. This designation indicates no hazardous laser energy is accessible to the user during normal operation nor while in a failure mode. During the course of doing service work on the CyAn ADP the interlock and other safety features may need to be disabled. If this is the case always wear safety laser goggles as well as personal protective equipment to protect your eyes and skin from laser light exposure.

Note The CyAn has been designed to operate as a Class 1 Laser Device. It must be operated with all light containment in place and all protective light seals intact.

Laser Hazard Classifications

The intent of laser hazard classification is to provide warning to users by identifying the hazards associated with the corresponding levels of accessible laser radiation with labels and instruction. It also serves as a basis for defining control measures and medical surveillance. Most of the U.S. domestic as well as all international standards divide lasers into four major hazard categories (I to IV) called the laser hazard classifications. The classes are based upon a scheme of graded risk and the potential to cause biological damage to the eye or skin.

Class I: cannot emit laser radiation at known hazard levels (typically continuous wave: cw 0.4 mw at visible wavelengths). Users of Class I laser products are generally exempt from radiation hazard controls during operation and maintenance (but not necessarily during service). Since lasers are not classified on beam access during service, most Class I industrial lasers will consist of a higher-class (high power) laser enclosed in a properly interlocked and labeled protective enclosure. In some cases, the enclosure may be a room (walk-in protective housing), which requires a means to prevent operation when operators are inside the room.

Class I.A.: a special designation that is based upon a 1000-second exposure and applies only to lasers that are “not intended for viewing” such as a supermarket laser scanner. The upper power limit of Class I.A. is 4.0 mW. The emission from a Class I.A. laser is defined such that the emission does not exceed the Class I limit for an emission duration of 1000 seconds.

Class II: low-power visible lasers that emit above Class I levels but at a radiant power not above 1 mW. The concept is that the human aversion reaction to bright light will protect a person. Only limited controls are specified.

Class IIIA: intermediate power lasers (cw: 1-5 mW). Only hazardous for intrabeam viewing. Some limited controls are usually recommended. **NOTE:** There are different logotype labeling requirements for Class IIIA lasers with a beam irradiance that does not exceed 2.5 mW/cm^2 (Caution logotype) and those where the beam irradiance does exceed 2.5 mW/cm^2 (Danger logotype).

Class IIIB: moderate power lasers (cw: 5-500 mW, pulsed: 10 J/cm^2 or the diffuse reflection limit, whichever is lower). In general Class IIIB lasers will not be a fire hazard, nor are they generally capable of producing a hazardous diffuse reflection. Specific controls are recommended.

Class IV: High power lasers (cw: 500 mW, pulsed: 10 J/cm^2 or the diffuse reflection limit) are hazardous to view under any condition (directly or diffusely scattered) and are a potential fire hazard and a skin hazard. Significant controls are required of Class IV laser facilities.

Eye Hazard

Due to the lens-like focusing effect of the human eye, it is 100,000 times more vulnerable to injury than the skin. Laser safety goggles should always be worn when working with an open beam. However, laser eye safety should be considered the last line of defense against laser beam exposure.

Remove all jewelry when working with an open beam.
When possible, use all protective housings, interlocks and shields.

When such defenses are not in place (during laser repair, alignment or installation) **EYE PROTECTION IS REQUIRED**.

Biological Effects of Laser Irradiation

Eye Injury. Because of the high degree of beam collimation, a laser serves as an almost ideal point source of intense light. A laser beam of sufficient power can theoretically produce retinal intensities at magnitudes that are greater than conventional light sources, and even larger than those produced when directly viewing the sun. Eye exposure to a direct beam can cause blindness.

Thermal Injury. The most common cause of laser-induced tissue damage is thermal in nature, where the tissue proteins are denatured due to the temperature rise following absorption of laser energy.

The thermal damage process (burns) is generally associated with lasers operating at exposure times greater than 10 microseconds and in the wavelength region from the near ultraviolet to the far infrared (315 nm-1030 nm.) Tissue damage may also be caused by thermally induced acoustic waves following exposures to sub-microsecond laser exposures.

Skin Hazard

To the skin, UV-A (315 nm-400 nm) can cause hyperpigmentation and erythema. Exposure in the UV-B (280 nm-315 nm) range is most injurious to skin. In addition to thermal injury caused by ultraviolet energy, there is the possibility of radiation carcinogenesis from UV-B. Exposure in the shorter UV-C (200 nm-280 nm) and the longer UV-A ranges seems less harmful to human skin than UV-B. The shorter wavelengths are absorbed in the outer dead layers of the epidermis (stratum corneum) and the longer wavelengths have an initial pigment-darkening effect followed by erythema if there is exposure to excessive levels.

The hazards associated with skin exposure are of less importance than eye hazards; however, with the expanding use of higher-power laser systems, particularly ultraviolet lasers, the unprotected skin of personnel may be exposed to extremely hazardous levels of the beam power if used in an unenclosed system design.

Basic Biological Effects of Laser Irradiation

Photobiologicalspectraldo main	Eye Effects	Skin Effects
Ultraviolet C (200 – 280 nm)	Photokeratitis	Erythema (sunburn), Skin cancer
Ultraviolet B (280 – 315 nm)	Photokeratitis	Accelerated skin aging, Increased pigmentation
Ultraviolet A (315 – 400 nm)	Photochemical UV cataract	Pigment darkening, Skin burn
Visible (400 – 780 nm)	Photochemical and thermal retinal injury	Photosensitive reactions, Skin burn
Infrared A (780 – 1400 nm)	Cataract and retinal burns	Skin burn

Electrical Hazard

Lethal discharge of the laser tube
Laser power supply produces lethal current
Keep laser cover on & interlocks operative
Never stand in pools of sheath/water with laser powered ON

Fire Hazard

Combustible materials placed in open (unsafe) beam path.

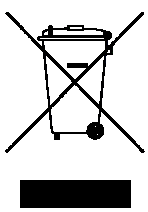
Biological Safety

1. Dako recommends that an appropriate disinfection agent be used in the Waste Tank.

2. The operator may be splashed/sprayed by the contents of the sample tube when it is changed. A risk analysis should be conducted to determine the appropriate OSHA-approved personal protective devices required.

3. As always, universal laboratory precautions should be adhered to. A ‘climate of safety’ is the single most important factor in the proper use and maintenance of the CyAn Analyzer.

The CyAn product line may be used for the analysis of pathogenic or other injurious agents. These operations can constitute a biohazard for the service representative. Dako does not certify any instrumentation for use with any hazardous organism or agent. Dako, strongly recommends explicit guidance be obtained from the institutional or company Biosafety Officer prior to operating this instrument with any potentially hazardous organism or agent. If any biohazardous organism or agent is used in the instrument, the user must inform Dako, in writing, prior to any Field Service visit or return of any part for service by Dako, or any of its vendors. Safety of the user as well as safety of all employees of Dako is of overriding importance.



Correct Disposal of this Product
(According to Directive 2002/96/EC on Waste Electrical and Electronic Equipment [WEEE] applicable in the European Union and other European countries with separate collection systems).

Contact a Dako representative for disposal of the equipment at the end of its working life. This product should not be mixed with other commercial waste for disposal.

Section 2

System Overview

The CyAn ADP™ is a state-of-the-art flow cytometer that utilizes one or more laser excitation sources to analyze biological cells, beads, or other microscopic particles as they are transported through an interrogation point in single file. Information can be gathered from large numbers of particles in a relatively short time so that populations can be studied or differentiated from other populations using simple to complex statistical methods. Physical and biochemical characteristics that can be measured using this instrument include but are not limited to forward scatter (size-related), side scatter (morphology-related), fluorescence from tagged (stained) cells/particles, and auto fluorescence from non-stained cells/particles.

CyAn ADP Subsystems

CyAn ADP has four key subsystems that form a powerful research tool. The four subsystems are: Fluidics, Optics, Electronics, and Software.

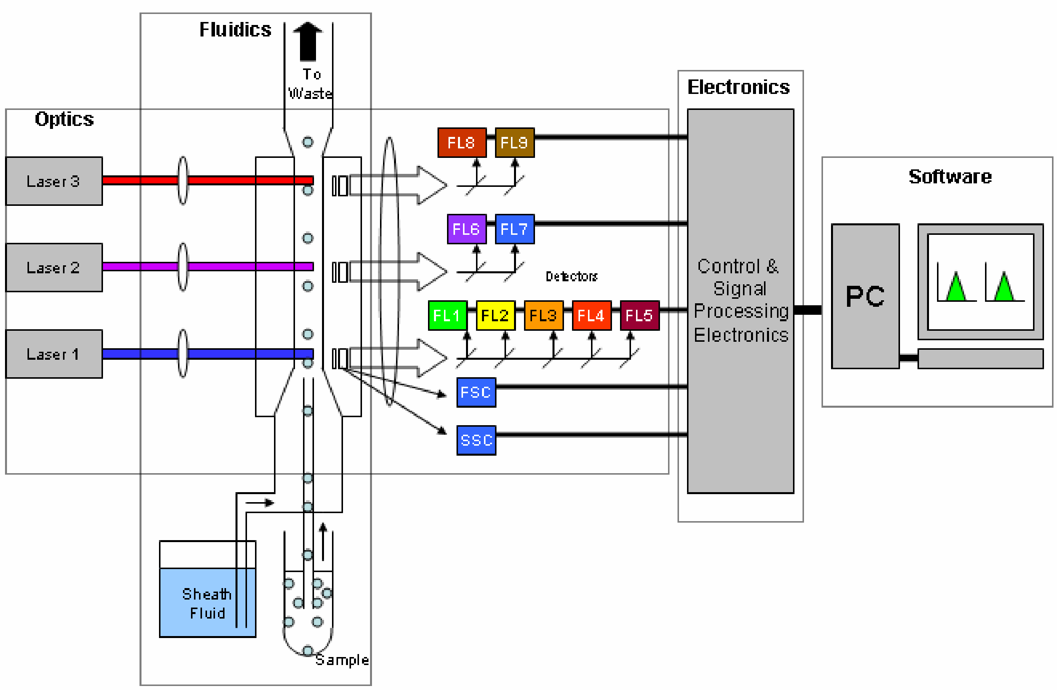


Figure 2.1: CyAn ADP Functional Block Diagram

A functional block diagram for the CyAn ADP High-Performance Flow Cytometer is shown in figure 2.1. The fluidics system pressurizes the system and transports particles to the interrogation point. Lasers are used as excitation sources and their beams, along with the ensuing scatter and fluorescence, are directed within the optical system. Electronics are used to power and control the instrument functions. The software provides the user interface for the above fluidics, optical, and electronic hardware, and the functions to acquire, analyze, and store data associated with the particles.

Fluidics

The Sheath Management System controls the transfer of sheath fluid, waste, and cleaner fluid throughout the CyAn ADP system. Sheath fluid is housed in either a replaceable 20 liter cubitainer or 20 liter plastic carboy. Waste is contained in a 20 liter plastic carboy. Cleaner fluid is contained in a 5 liter cubitainer. The Sheath Management System provides improved sheath pressure stability and prevents bubbles from entering the system when the sheath container is changed. The built-in cleaner fluid cubitainer allows the user to easily clean and rinse the sheath path using Dako Clean and Rinse solution and sheath or DI water as rinsing fluid. The 20 liter sheath and waste containers provide approximately 24 hours of system run time.

Figure 2.2 outlines the key fluidics components of CyAn ADP. In general, flow of air and fluids follows a path left to right in the diagram. An air compressor provides pressure for propulsion of the sample, cleaner, and sheath fluids. Air regulators condition and stabilize the pressure source prior to sheath and cleaner reservoirs and sample vessel. A number of electrically controlled valves control the flow state of the system and provide a means for cleaning and debubbling, in addition to routine flow conditions for sample analysis.

Sample is forced through tubing, is introduced to the flow cell, and is fluidically focused by sheath fluid. This hydrodynamic focusing effect causes individual particles to be introduced in single file to each of the sequential laser beams. While a pinch valve is used to allow the flow of sample, the rate of sample flow is controlled by adjusting the over-pressure (differential pressure) of an electrically controlled air regulator relative to sheath pressure. Waste is drawn by a vacuum pump to a holding container for disposal.

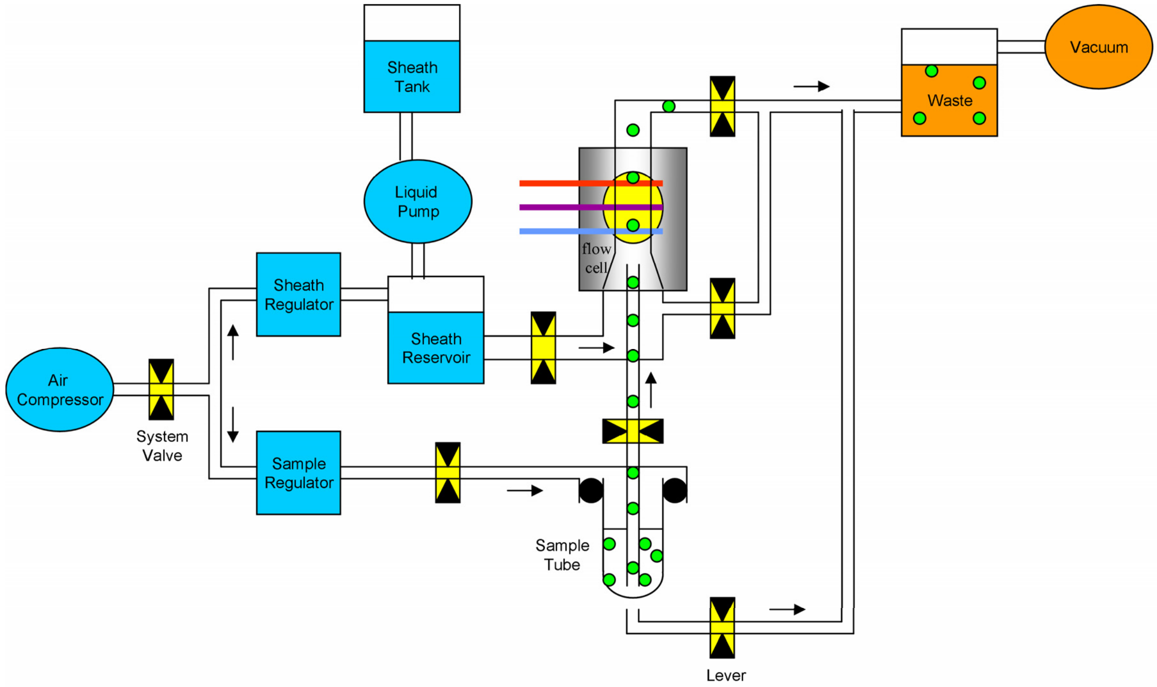


Figure 2.2: CyAn ADP Fluidics Block Diagram

Optics

Figure 2.3 is an illustration of the optical geometry of the CyAn ADP UV model. Up to three excitation sources can be accommodated on the optical bench. Each path has its own independent, unique steering and focusing elements to provide optimal excitation of particles at the interrogation point. Shown in the figure are laser paths for a 488 nm line (blue), a UV line and a 635 nm line (red). Each laser beam is focused to the quartz flow cell, where particles are transported past the laser beams.

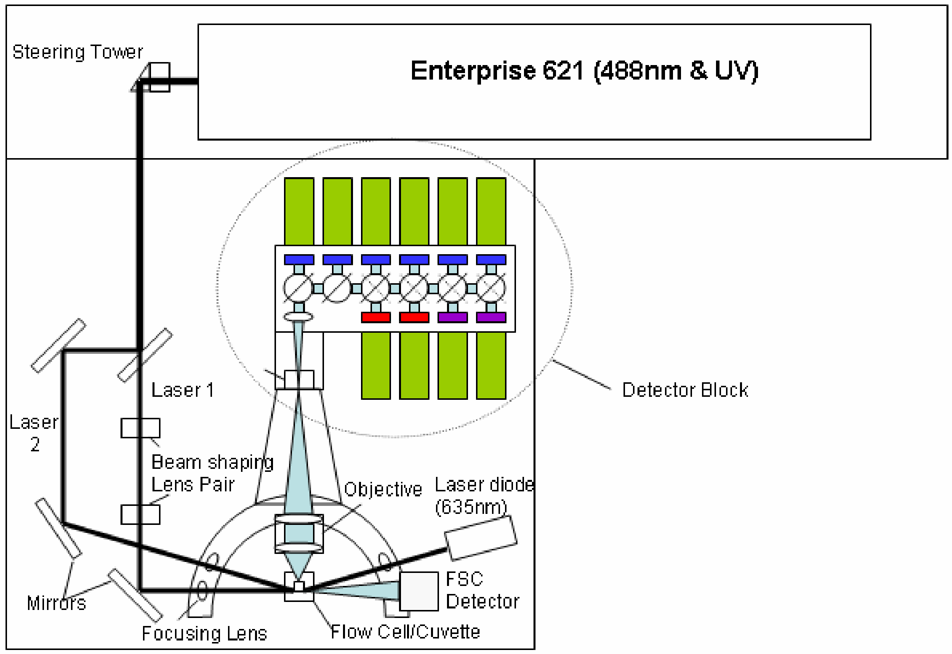


Figure 2.3: CyAn ADP UV Model Optical Block Diagram and Location of Laser Apertures

The focused beams are separated vertically to ensure minimal optical crosstalk between detection paths. Further, the signals that are detected as particles that traverse the beams are electronically gated to reduce unwanted signal interference between channels. A high numerical aperture microscope objective is used to collect scatter and fluorescence and to generate an image of the interrogation point for each of three apertures (spatial filters). The apertures improve signal to noise by preventing unwanted scatter around the excitation region from entering the detector block. Light that transmits through the spatial filters is reflected and spectrally filtered on its way to the appropriate photomultiplier tubes (PMT) for detection.

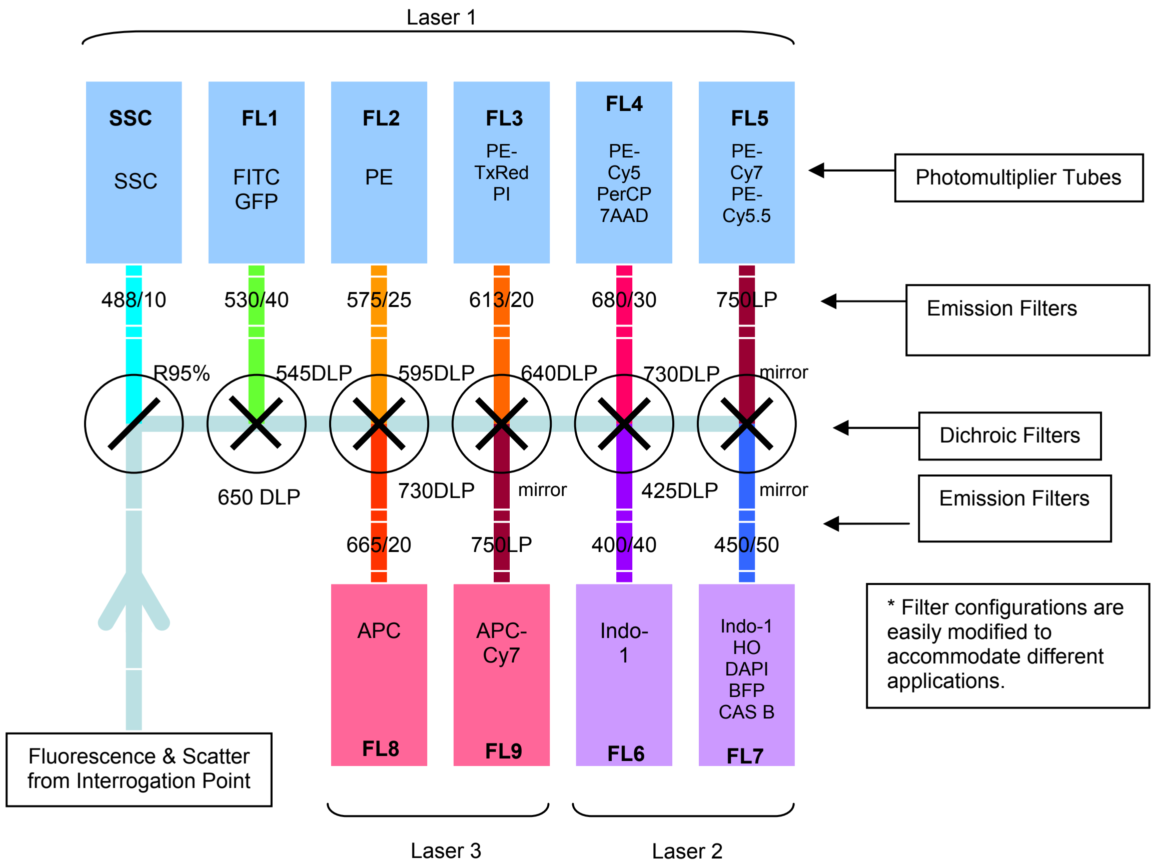


Figure 2.4: CyAn ADP UV Model Detector Block and Optical Filter Layout

Figures 2.4 and 2.5 illustrate the location, position, and orientation of the optical filters and PMTs within the detection block assembly of the CyAn ADP UV Model and CyAn ADP respectively. Dichroic filters are located at 45° to the direction of light propagation, while emission filters are located at 90° to the light path. Filter sticks are interchangeable, thus allowing custom configurations to be implemented. Please contact Dako to order dichroic filter sets.

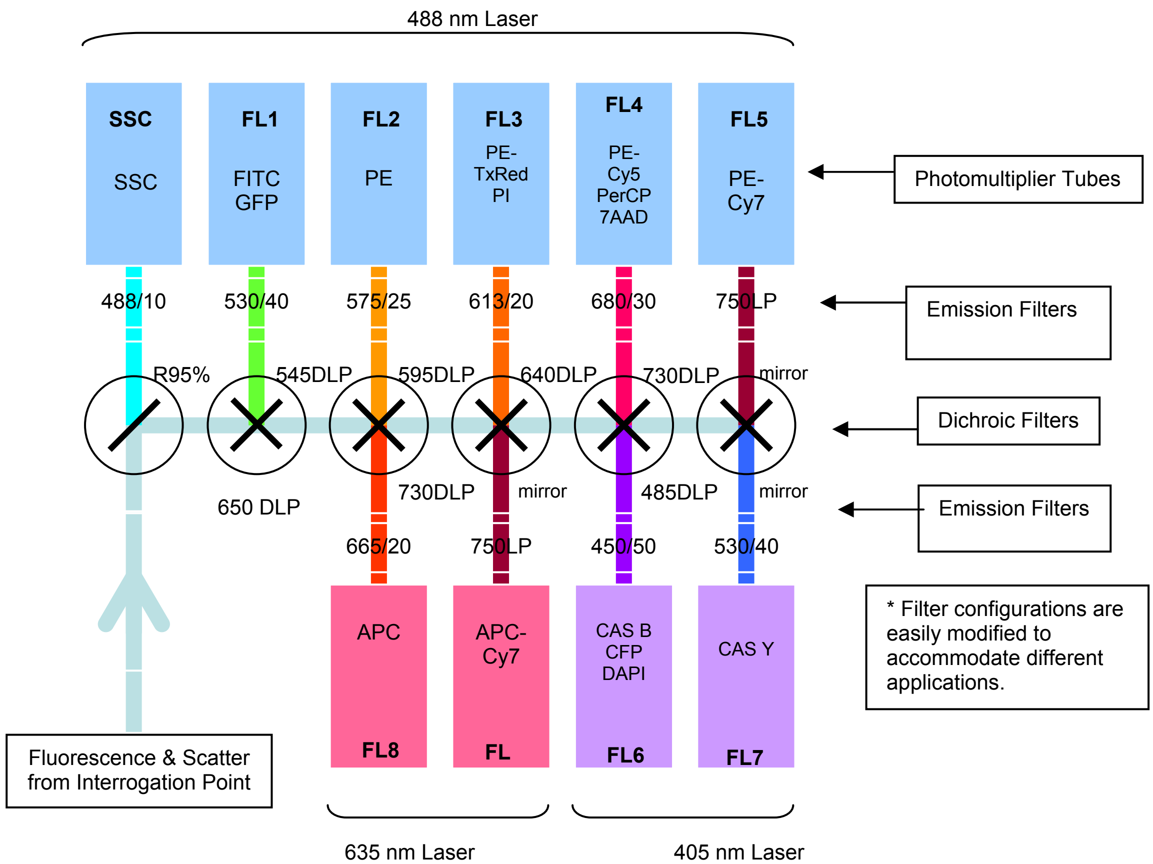


Figure 2.5: CyAn ADP Detector Block and Optical Filter Layout

Electronics

CyAn ADP has an array of electronic components. The PC/workstation is used for instrument control, status, and data acquisition functions and communicates with the CyAn ADP instrument through a high-speed serial link. Housed within the instrument is a state-of-the-art electronic chassis that provides a communication backplane/bus with a number of available card slots to allow modular connection of key electronic control and sensing components. These components include trigger and signal processing, and multi-function input/output. Peripheral to the electronic chassis, but within the instrument are a number of devices that can be grouped as fluidics control and sense (pumps, regulators, valves, and sensors), laser/shutter control and sense, PMT voltage control, and PMT signal. The multifunction I/O card is used for controlling these peripheral devices and sensing instrument status. The PMT and photodiode detectors convert light emitted from particles excited at the interrogation point, into electrical signals. These signals are input to the trigger and signal processing cards. Amplification, analog to digital conversion, and sampling techniques provide quantitative measurements including peak, area/integral, log, and pulse width for a given triggered particle event.

Peripheral Devices

The CyAn ADP instrument has the following peripheral devices: Summit Workstation, two Monitors, a printer, and Sheath Management System (SMS). The SMS houses sheath container, waste container, cleaner cubitainer, level indicators, in-line sheath filter, and compressor/vacuum pump.

Software

CyAn ADP uses Summit Data Acquisition and Analysis Software for instrument control, data acquisition, and subsequent data analysis. Summit is the Windows user interface for the entire Dako flow cytometry product line.

Summit software offers users complete control of the CyAn ADP instrument at varying levels of complexity, depending on their needs. The instrument control panel (figure 2.6) provides access to laser control, event rate settings, system maintenance functions such as clean and rinse, and Sheath Management System functions. The sample parameters panel (figure 2.7) has software controls for adjusting parameter settings such as threshold, PMT voltage, and gain.   User documentation for Summit is available from the **Help** menu.

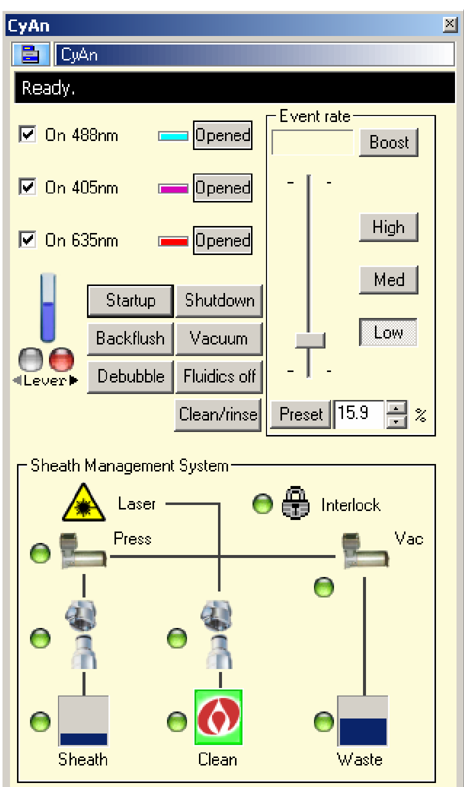


Figure 2.6: Instrument Control Panel

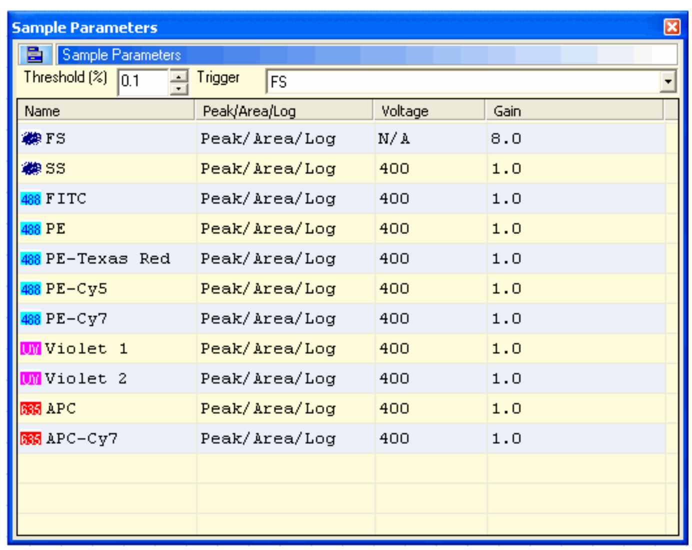


Figure 2.7: Sample Parameters Panel

Section 3

System Specifics

CyAn Overview

This section will provide specific information related to the three main components of the CyAn ADP: the Sheath Management System (SMS), the CyAn instrument, and the Summit Workstation.

Sheath Management System (SMS)

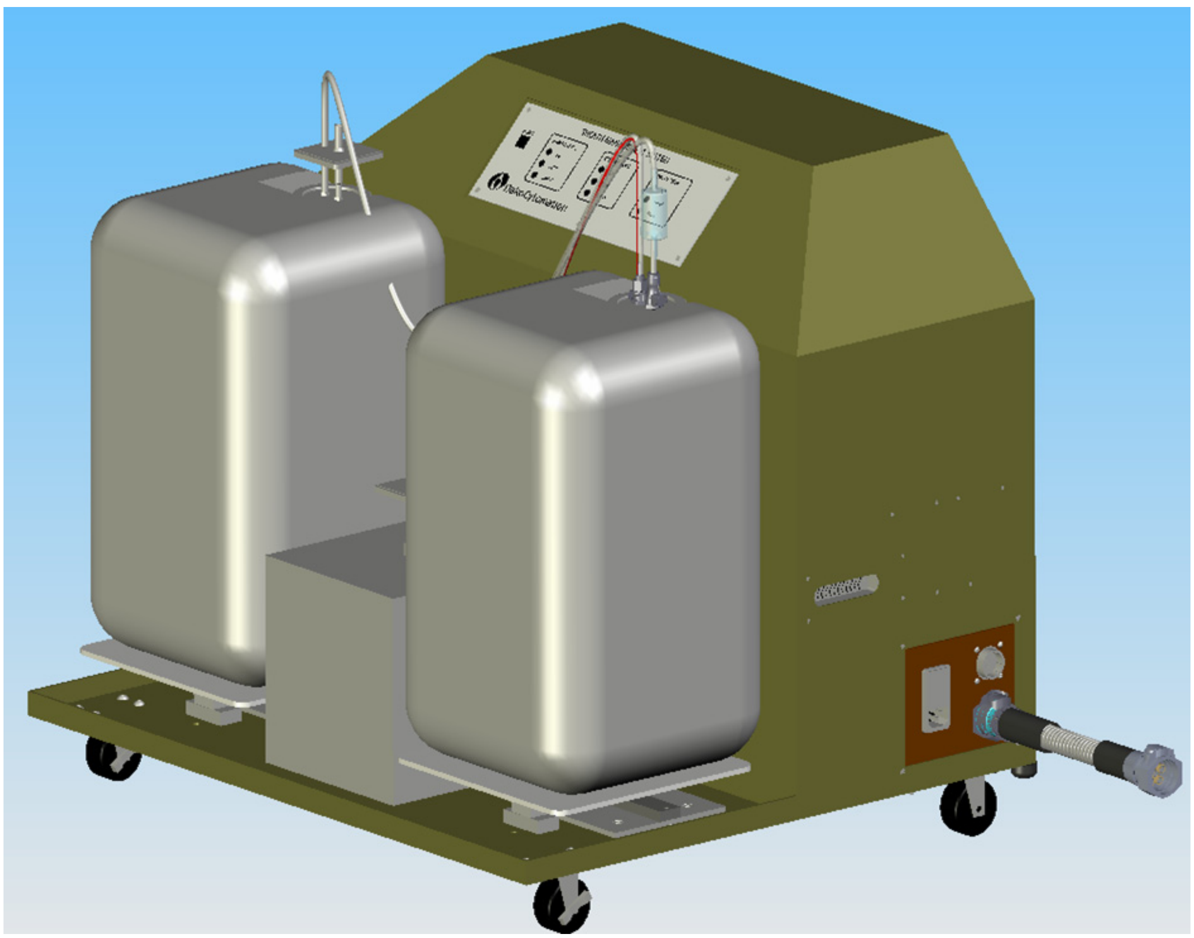


Figure 3.1: SMS Drawing

Figure 3.2: SMS front view with reusable sheath and waste tanks and Dako cleanser box.

SMS Fluidic Theory of Operation

The goals of the Sheath Management System are to provide:

Sheath fluid pushed with a stable pressure
Waste fluid and air pulled with sufficient flow to allow vents to open air
Ability to have unpressurized sheath, cleaner, and waste containers
Filtering of sheath and cleaner to remove any contaminants
General sheath and waste container fluid level information
Software control of the fluid system
Ability to catch most errors

Sheath fluid pushed with a stable pressure

Air Supply
The SMS has three air regulators, with each feeding the input of the next. This should result in a sheath air pressure that is very accurate and consistent.

Liquid Level
Once this air pressure is applied to the internal reservoir, the liquid level is the next piece that can affect the stability of the Sheath provided. The reservoir has three float switches in it. One switches when the reservoir is overfilled. One shows when the reservoir is empty. The middle switch is used in normal operation to control the liquid level height. When the switch is off, the liquid pump will run until the switch turns back on. This hysteresis of the switch determines the height variation of the liquid level.

Liquid Pumping
There is also a small variation of the pressure when the liquid pump turns on to refill the reservoir. There is documentation to describe the amount, but it is very small. The reservoir is refilled approximately every 30 seconds when in normal sheath mode.

Temperature
In testing it has been found that the temperature of the lab makes a significant difference in the Sheath pressure that is supplied. It may be an advantage for a customer to have better environmental control, or move the system to a location that has more stable temperature.

Waste fluid and air pulled with sufficient flow to allow vents to open air

The SMS has an oversized waste pump that can handle both liquid and air. It can provide sufficient vacuum pressure.

Ability to have unpressurized sheath, cleaner and waste containers

With pumps connected to the sheath, cleaner, and waste external containers, the containers can be un-pressurized. The waste container may experience a small positive pressure as air is pushed out the exhaust filter. The Sheath and Cleaner containers may experience a small negative pressure as air is pulled into the container.

Filtering of sheath and cleaner to remove any contaminants

The sheath filter is after the sheath reservoir and the cleaner filter is before the cleaner liquid pump.

General sheath and waste container fluid level information

Load cells that can be tuned with the SMS control board, are read by software. They are compared to saved values in the software to decide when early warnings, Low and Empty, should be displayed, and for waste when the system should be stopped.

Software control of the fluid system
The SMS Control Board gets all of its control signals from the CyAn Control Board. All functions except a hard reset can be controlled by the software. The SMS can be turned on and off, all states controlled, and errors can be reset.

Ability to catch most errors

A more comprehensive document with error codes and the results should be consulted for more detailed information.

Waste System:
Too high of pressure in the Waste Container - Waste filter may be clogged or waste container is over filled.
Not enough Vacuum pressure created - Too much fluid in the pump or too big of an opening to ambient. For instance, during debubble there is a slug of fluid reaching the waste pump.

Air System:
System pressure too low - Not enough air supply or an air leak in pneumatics.
Sheath pressure too low - Not enough air supply or an air leak in pneumatics.

Liquid System:
Pump vacuum pressure too high - Indicates blockage in the path to the liquid container or quick connectors are disconnected.

Reservoirs:
Overfilled - Fluid level in the reservoir is too high
Low - Tell the pumps to prime to bring the level back up (normal operation)
Empty- The level in the reservoir has dropped below the lowest switch.

Power Supplies:
Three checks to make sure the power supply is working correctly.

External Containers:
Software controls the use of the load cell feedback.
Time warning - warning when there is approximately this percentage of sheath.
Low - load has reached the low setting- Sheath Container.
High - load has reached the full setting- Waste Container.
Full for waste - the waste subsystem will halt when the waste gets too full.

Path of Sheath and Cleaner

Liquid Path

External Sheath Container to Internal Reservoir
The sheath starts in a non pressurized container, either a cubitainer or a reusable container. A small liquid pump pulls sheath past a vacuum switch and into an internal pressurized reservoir. The vacuum switch will cause an error when the vacuum becomes too great indicating that the sheath flow to the pump has been restricted or stopped. As the sheath is pulled out of the container, if it is a reusable container, air is allowed to enter through an air filter. If the container is a cubitainer then it contains a collapsible bag that does not need to let any in any air.

External Cleaner Container to Internal Reservoir
The cleaner goes through the same series of steps as the Sheath. One difference is that it has its own pressure switch, its own internal reservoir, and is only available with a Cubitainer. The other difference is that the cleaner goes through a filter before it goes into the liquid pump. The cleaner cap does have an air filter to allow air into the cleaner Cubitainer.

Pressure Reservoir
The two pressure reservoirs are identical to each other. The have a connection for the liquid to be pumped in. This connection also has an in-line check valve so that no liquid will flow into the container when the pumps are off. There is a pressure inlet that pressurizes the reservoir to push the liquid. The last of the tubing connections is a tube that reaches into the reservoir to deliver the liquid to the CyAn. There are also two air release valves. One is an over pressure valve that will bleed off pressure if it rises too high. The second is a manual bleed off to release the pressure held in the reservoir if needed for service. There are also three level float switches. These watch for errors, and keep the level of the liquid constant.

Sheath to CyAn
From the pressure reservoir the sheath goes into the Sheath Filter Assembly. Here it goes through the Sheath filter. The Sheath filter has a valve that allows the top of the sheath filter to be connected to waste which clears out any air in the filter. The sheath then goes through a selection valve that decides if sheath or cleaner will flow to the CyAn. Just past this selection valve is the cutoff valve that stops liquid flow to the CyAn. This valve allows the SMS to maintain pressure when it is not in a sheath flow mode, and be instantly ready to provide sheath when needed. Liquid flows from here to the Fluidic Hose, and then up to the CyAn.

Cleaner to CyAn
From the pressure reservoir the cleaner goes directly to the selection valve on the Sheath Filter Assembly. When the valve is in cleaner mode the fluid will flow the same way the sheath did through the Fluidic Hose and up to the CyAn.

Air Path

Air Pressure Path

Air comes into the pressure reservoir from two coalescing filters one for Sheath and one for Cleaner. These are designed to remove oil and particles and to help prevent vapor from the reservoirs from exhausting through the regulators when the system is de-pressurized. These two lines combine and then enter the Air Regulator Assembly. The first thing that they encounter is a switching valve that decides if the air pressure should be high or low. If it is low the pressure comes from the third regulator (Sheath), if it is High the pressure will come from the second regulator (High Pressure). The Low Pressure regulator is supplied from the output of the high pressure regulator. There is also a pressure switch on the output of the Low Pressure regulator to make sure that a minimum pressure is supplied. The High Pressure regulator gets pressure from the first regulator (System). The System regulator has a pressure switch that makes sure that the system pressure doesn’t drop too low. This regulator is supplied from the Air Dryer Assembly.

Air Dryer Path

Air going to the regulator assembly comes from the membrane dryer. This dryer separates out the water vapor from the compressed air coming from the air compressor. The vapor that is removed is exhausted into the SMS shell. The inlet of the membrane dryer is connected to the main coalescing filter. This filter cleans out an oil or debris that may come out of the air compressor. The bottom of the coalescing filter has a high pressure bleed off valve that should be bleeding off slightly in normal operation. This valve ensures that the pressure going into the system does not get too high. It also serves to remove any moisture that collects in the filter to drain out the drain hole in the bottom of the SMS.

Air Compressor

The inlet of the main coalescing filter is connected to the air compressor. The compressor pulls in air from a long tube that reaches toward the air inlet to the SMS. This is intended to grab air from the outside environment instead of the warmer air inside the cabinet and reduces noise.

Waste Path

Waste Pump

The lines coming into the waste pump are the CyAn waste, the sheath filter debubble, and the waste vacuum sensor. The waste vacuum sensor makes sure that there is enough vacuum pressure to keep the CyAn overflow reservoir empty.

External Waste Container

The waste goes through the waste pump and out to the external waste container. The air is pushed through the waste filter to be cleaned before it is allowed into the environment.

There is also a waste container pressure switch. This switch is in place to make sure that the positive pressure in the waste container does not get too high. Pressure can build up in the waste container if the waste filter is plugged up or full of liquid.

SMS Assemblies

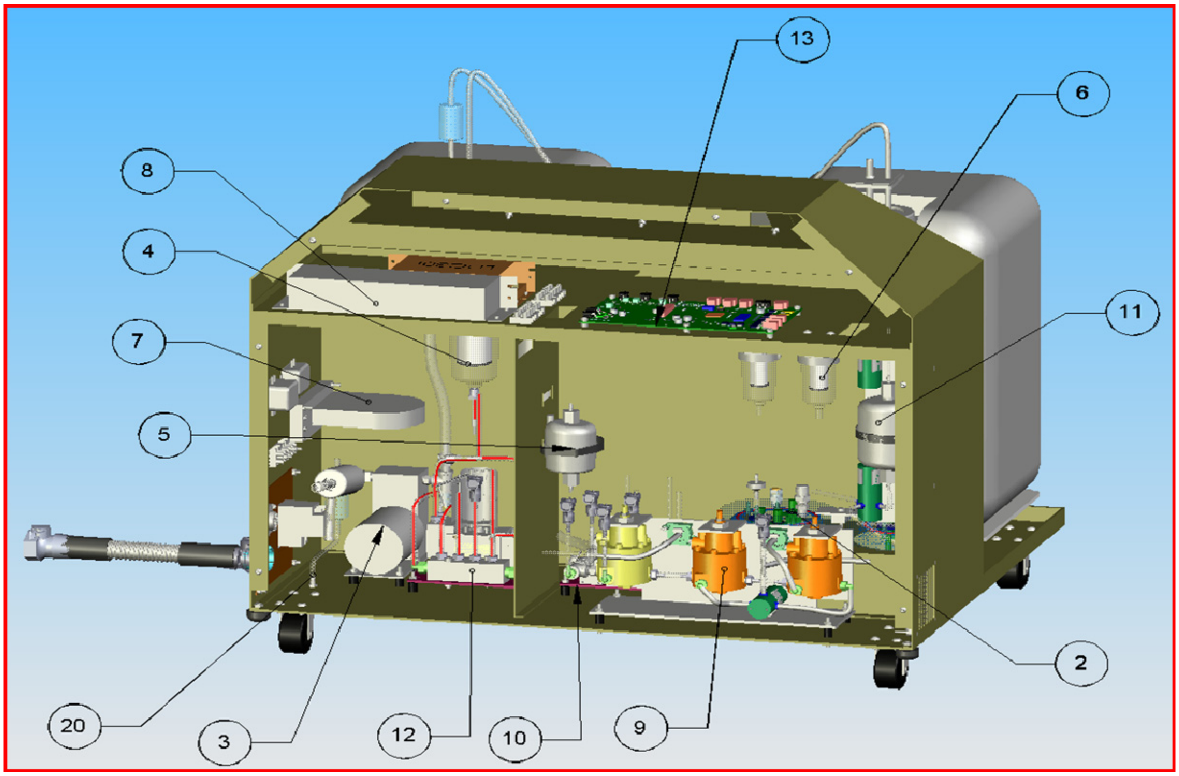


Figure 3.3: Rear view of SMS with panel removed.

1	SMS Main Cabinet Assy	11	SMS Sheath Filter Assy
2	SMS Sheath/ Clean Pressure Reservoir Assy	12	SMS Waste Pump Assy
3	SMS Air Compressor Assy	13	SMS Control Board
4	SMS Air Dryer Assy	14	SMS Load Cell Assy
5	SMS Cleaner Filter Assy	16	SMS Sheath Container Assy
6	SMS Coalescing Filters Assy	17	SMS Waste Container Assy
7	SMS Turbine Fan Assy	18	SMS Cleaner Container Assy
8	SMS Power Supply Assy	19	SMS Bulkhead Assy
9	SMS Air Regulator Assy	21	SMS Fluidics Hose Assy
10	SMS Sheath and Clean Pump Assy	23	SMS Waste Container Connection Assy

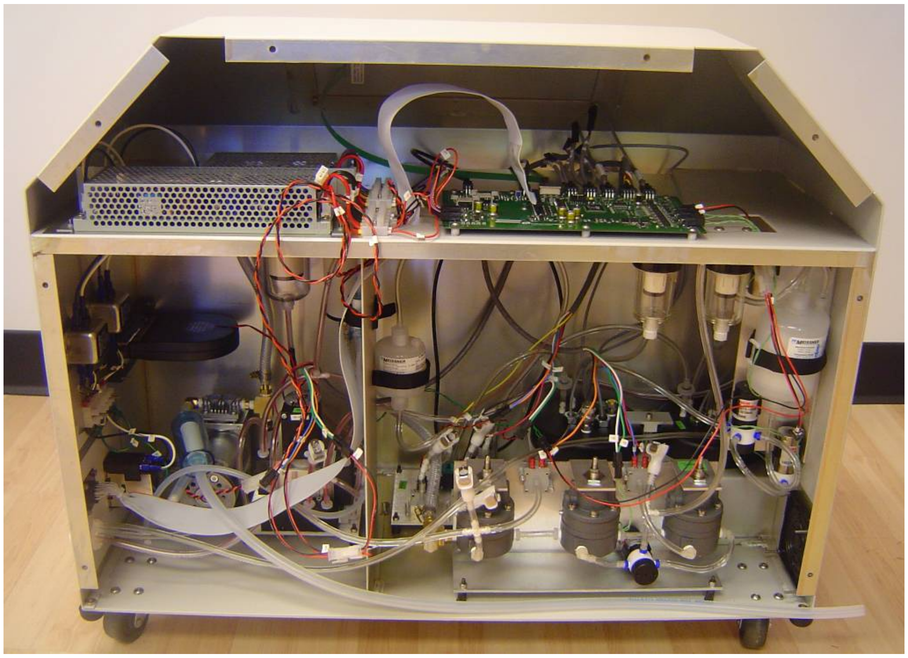
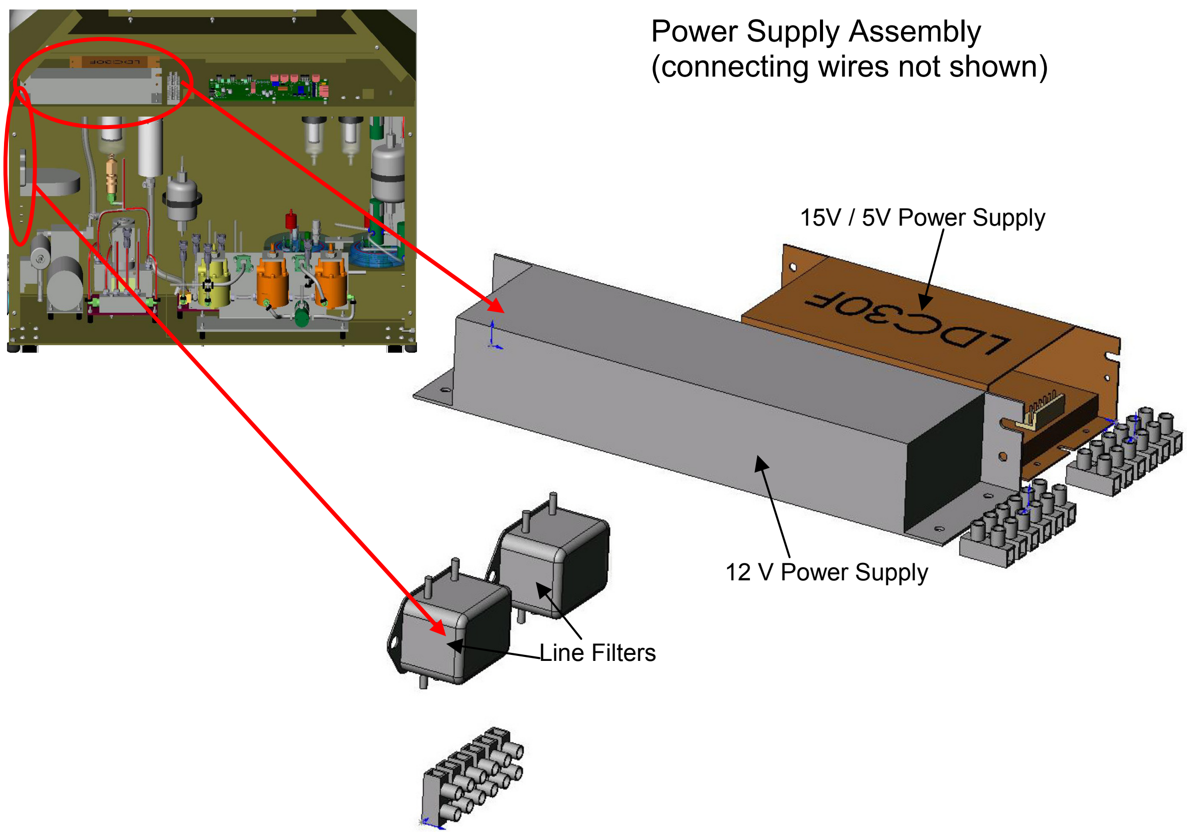
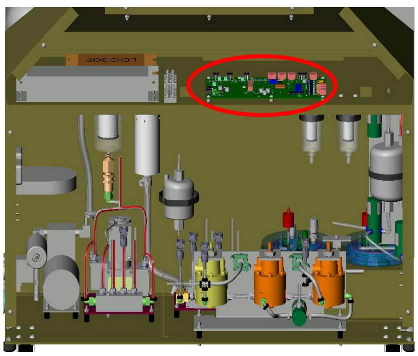


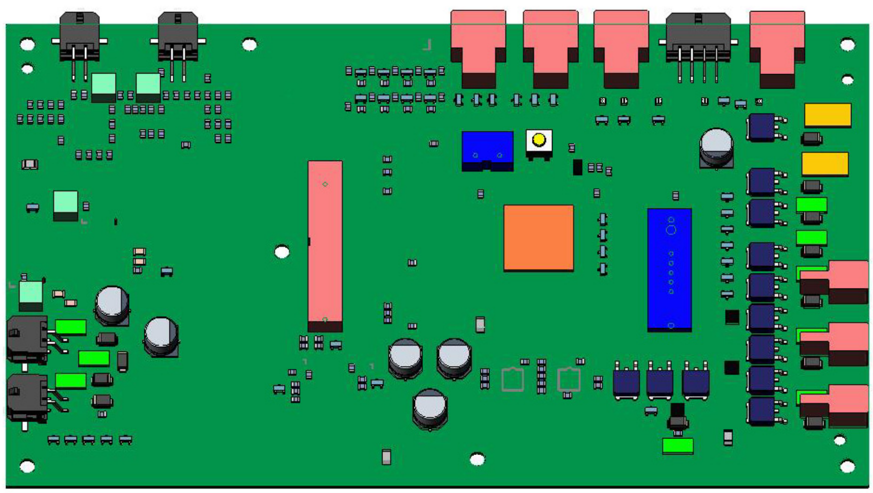
Figure 3.4: Picture of SMS from rear with panel removed.



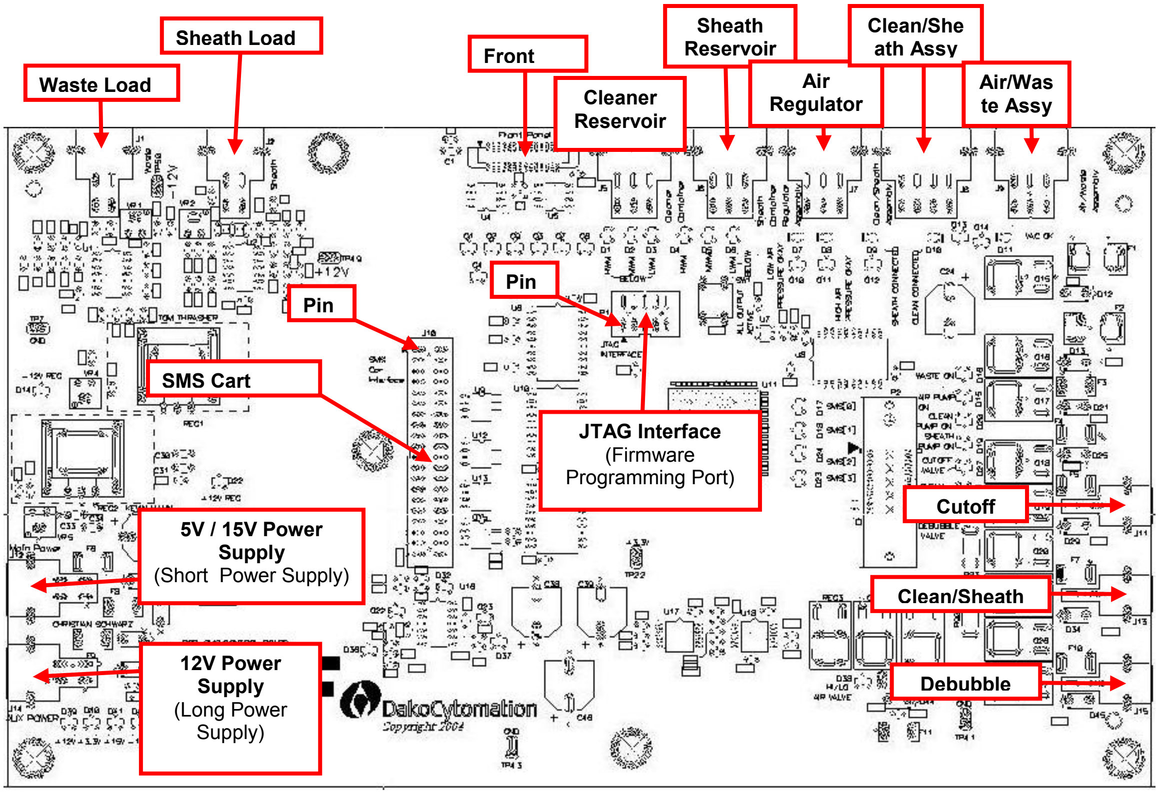


SMS Control Board

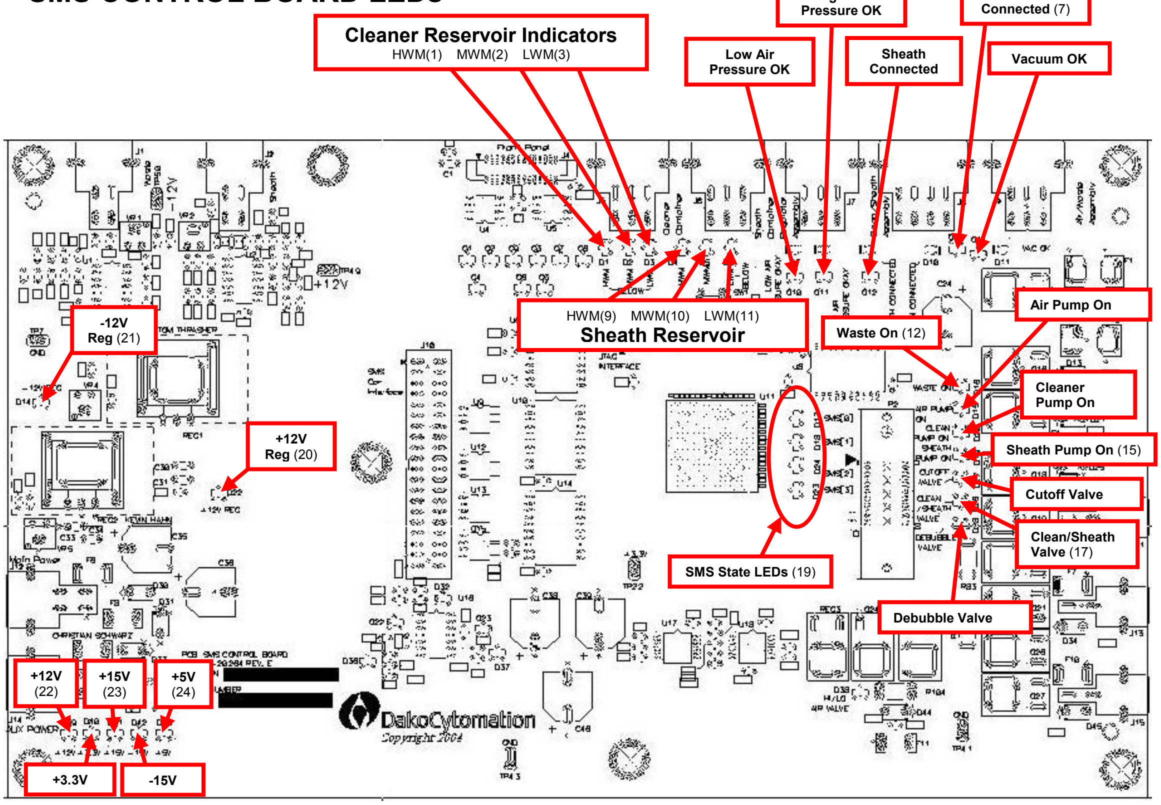
For more information, see the SMS Control Board Layout Document



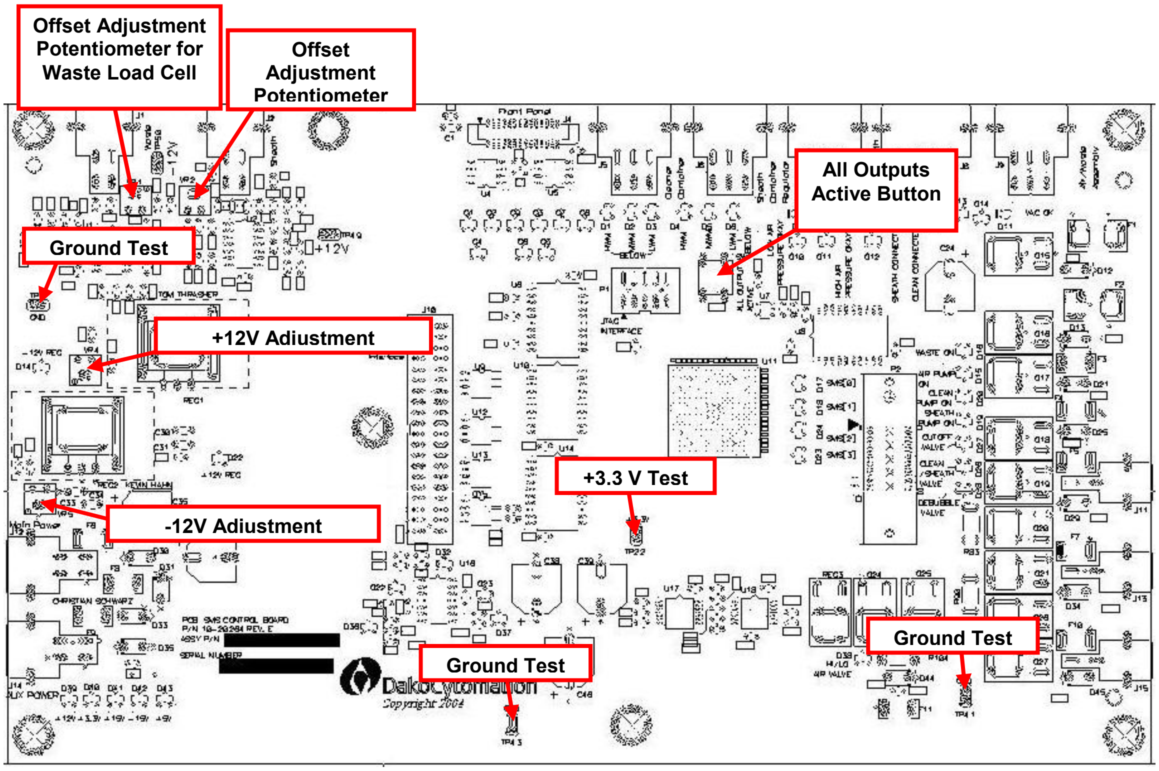
SMS CONTROL BOARD CONNECTIONS



SMS CONTROL BOARD LEDs



SMS CONTROL BOARD MISCELLANEOUS



SMS Control Board Error Light Conditions

LED	Normal Operating Conditions	Error Conditions	Related Summit Error Codes
Cleaner Reservoir HWM	Green	Not illuminated – This occurs when the cleaner reservoir is overfilled.	305, 307
Cleaner Reservoir MWM	Not illuminated / Red – This LED is illuminated while the reservoir is being refilled.	Red – If the LED is illuminated for longer than XXX seconds, the cleaner reservoir is low and cannot be refilled. This is usually due to the external container being empty.	301
Cleaner Reservoir LWM	Not illuminated	Red – If the LED is illuminated, the liquid has reached the low water mark. The SMS will be turned off XXX seconds after this LED is illuminated.	302
Low Air Pressure	Green	Red – This LED will not be illuminated when the SMS is off or if the air regulator assembly has detected an error.	997
High Air Pressure	Green	Red – This LED will not be illuminated when the SMS is off or if the air regulator assembly has detected an error.	997
Sheath Connected	Green	Red – This LED	404

LED	Normal Operating Conditions	Error Conditions	Related Summit Error Codes
		will turn red when the sheath quick connect has become disconnected.	
Cleaner Connected	Green	Red – This LED will turn red when the cleaner quick connect has become disconnected.	304
Vacuum OK	Green	Red – This LED will turn red if the waste pump assembly detects a loss of pressure or an overpressure in the waste tank.	998
Sheath Reservoir HWM	Green	Not illuminated – This occurs when the sheath reservoir is overfilled.	405, 407
Sheath Reservoir MWM	Not illuminated / Red – This LED is illuminated while the reservoir is being refilled.	Red – If the LED is illuminated for longer than XXX seconds, the sheath reservoir is low and cannot be refilled. This is usually due to the external container being empty.	401
Sheath Reservoir LWM	Not illuminated	Red – If the LED is illuminated, the liquid has reached the low water mark. The SMS will be turned off XXX seconds after this LED is illuminated.	402

SMS Error Conditions and Status

The following information describes SMS errors (which appear in the CyAn Control Panel as tool tip text) along with specific Summit behaviors, SMS subsystem states, and CyAn status.

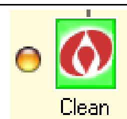
Error Condition	**Message 101: The 621 Laser has reported a fault. Please check the cooling lines and interlocks on the 621 Laser.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit Control
SMS Indicator Behavior	N/A
SMS State (Normal means anything but Standby – 0000)	Non error state
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	Fault (1)
Service Plug In	N/A

Error Condition	**Message 102: CyAn cover is open. The laser shutters have been closed and fluidic system is shutdown. Please close your cover and restart up.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None

SMS – Summit Expected Behavior	Summit Control
SMS Indicator Behavior	N/A
SMS State (Normal means anything but Standby – 0000)	Non error state
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 202: Summit has detected the service mode dongle. Warning: By lifting the cover you can now be exposed to direct laser light!**
Send to Error Log?	Yes
Priority of Message (0-10)	3
Summit GUI Indicator Behavior	N/A
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit Control
SMS Indicator Behavior	N/A
SMS State (Normal means anything but Standby – 0000)	Non error state
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior (no software action taken now)
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)

Error Condition	**Message 202: Summit has detected the service mode dongle. Warning: By lifting the cover you can now be exposed to direct laser light!**
Service Plug In	Present (1)

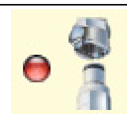
Error Condition	**Message 301: Cleaner fluid is low. Please replace the cleaner solution and press the Startup button on the CyAn Control Panel.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Cleaner low (amber)
SMS State (Normal means anything but Standby – 0000)	Clean
Sheath Subsystem State	Non error state
Clean Subsystem State	001 Reservoir low
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	When Clean and Rinse first starts, if Cleaner is low, then the Clean & Rinse Cycle will not start. Otherwise, the error is ignored during the clean and rinse cycle.
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 302: Cleaner fluid is empty. Please replace the cleaner solution and press the Startup button on the CyAn Control Panel.**

Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	Notify user of incomplete clean procedure if in clean and rinse cycle.
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover.
SMS Indicator Behavior	Cleaner empty (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Clean
Sheath Subsystem State	Non error state
Clean Subsystem State	010 Reservoir empty
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Skip to rinse if in clean and rinse cycle. Notify at end of cycle. Block user from doing another clean cycle until reservoir is filled.
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**No message**
Send to Error Log?	No
Priority of Message (0-10)	None
Summit GUI Indicator Behavior	N/A
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	None
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	011 System priming

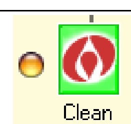
Error Condition	No message
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	Message 304: Cleaner quick connect is not completely engaged. Please check your connection.
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	Notify user of incomplete clean procedure
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Cleaner (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	100 Quick Con. Error
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Skip to rinse if in clean and rinse cycle. Notify at end of cycle. Block user from doing another clean cycle until reservoir is filled.
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 305: Internal reservoir overfilled. This will not prevent operation of the instrument but may require future service.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	One-time dialog that can be cleared by user
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Cleaner (amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	101 reservoir overfilled
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 306: Clean subsystem is halted. Please check that you have sufficient cleaner fluid.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	Notify user of incomplete clean procedure if in clean and rinse cycle
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover

SMS Indicator Behavior	Cleaner (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	110 system halted
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Skip to rinse if in clean and rinse cycle. Notify at end of cycle. Block user from doing another clean cycle until reservoir is filled.
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 307: Cleaner subsystem switch error. This will not prevent operation of the instrument but may require future service.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Cleaner (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	111 subsystem error
Waste Bit	Non error state

Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Skip to rinse if in clean and rinse cycle. Notify at end of cycle. Block user from doing another clean cycle until reservoir is filled.
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

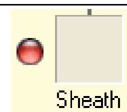
Error Condition	**Message 430: Less than 30 min of sheath fluid is remaining.**
Send to Error Log?	Yes
Priority of Message (0-10)	3
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Sheath low (amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	30 min.
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 410: Less than 10 minutes of sheath fluid is remaining. Please replenish your sheath fluid.**

Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Sheath low (amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	10 min.
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

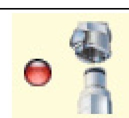
Error Condition	**Message 401: Internal sheath reservoir level is low. CyAn will stop soon. Please replenish your sheath fluid.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Sheath low (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	001 Reservoir low
Clean Subsystem State	Non error state
Waste Bit	Non error state

Error Condition	**Message 401: Internal sheath reservoir level is low. CyAn will stop soon. Please replenish your sheath fluid.**
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 402: Out of sheath fluid. Replenish sheath and press the Startup button on the CyAn Control Panel.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Sheath empty (red)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	010 Reservoir empty
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**No message**

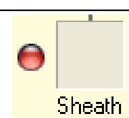
Error Condition	**No message**
Send to Error Log?	No
Priority of Message (0-10)	None
Summit GUI Indicator Behavior	N/A
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	None
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	011 System Priming
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 404: Sheath quick connect is not completely engaged. Please check your connection.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Sheath (flashing red)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	100 Quick Con. Error
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A

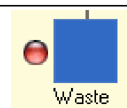
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Summit goes to fluidics shutdown state
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

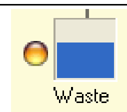
Error Condition	**Message 405: Internal reservoir overfilled. This will not prevent operation of the instrument but may require future service.**
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Sheath (amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	101 Reservoir overfilled
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

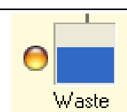
Error Condition	Message 406: Sheath subsystem is halted. Please check that you have sufficient sheath fluid.
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS Control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Sheath (red)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	110 System halted
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	Message 407: Sheath subsystem switch error. Service maybe required.
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS Control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Sheath (flashing red)
SMS State (Normal means anything but Standby –	Normal

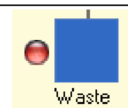
0000)	
Sheath Subsystem State	111 Subsystem error
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 705: Waste subsystem is halted. Please check waste tank level**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS Control – subsystem halted – requires reset to recover
SMS Indicator Behavior	Waste (red)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	Message 730: Less than 30 min until the waste container is full.
Send to Error Log?	Yes
Priority of Message (0-10)	3
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Waste (amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	30 min.
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

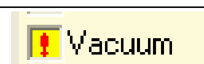
Error Condition	Message 710: Less than 10 min until the waste container is full. Please empty the waste container.
Send to Error Log?	Yes
Priority of Message (0-10)	2
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Waste (flashing amber)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state

Error Condition	Message 710: Less than 10 min until the waste container is full. Please empty the waste container.
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	10 min.
Summit Operation Expected Behavior	Normal behavior
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	Message 700: Waste container is full. Please empty the waste container.
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	Summit control
SMS Indicator Behavior	Waste (red)
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	Full
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

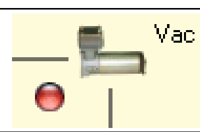
Error Condition	Message 905: Low sheath pressure inside CyAn. Check connection between the CyAn and SMS.

Error Condition	**Message 905: Low sheath pressure inside CyAn. Check connection between the CyAn and SMS.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – system shutdown
SMS Indicator Behavior	N/A
SMS State (Normal means anything but Standby – 0000)	Non error state
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	Error
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	**Message 906: Low vacuum inside CyAn. Check connection between CyAn and SMS.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – system shutdown
SMS Indicator Behavior	N/A
SMS State (Normal means anything but Standby – 0000)	Normal
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state

Error Condition	**Message 906: Low vacuum inside CyAn. Check connection between CyAn and SMS.**
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	Error
621 Laser Fault	No fault (0)
Service Plug In	N/A

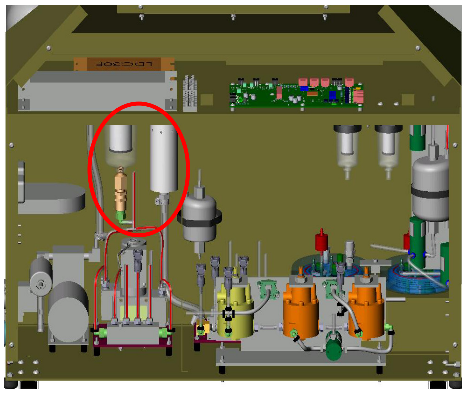
Error Condition	Message 997: Low sheath pressure in SMS.
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – system shutdown
SMS Indicator Behavior	Sheath (flashing red)
SMS State (Normal means anything but Standby – 0000)	1101 SMS int. error
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	N/A
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

Error Condition	Message 998: Waste subsystem halted due to lose of vacuum. If during operation, please check vacuum pump, waste quick connect or waste tank level.
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – system shutdown
SMS Indicator Behavior	Waste (flashing red)
SMS State (Normal means anything but Standby – 0000)	Not available from SMS state now

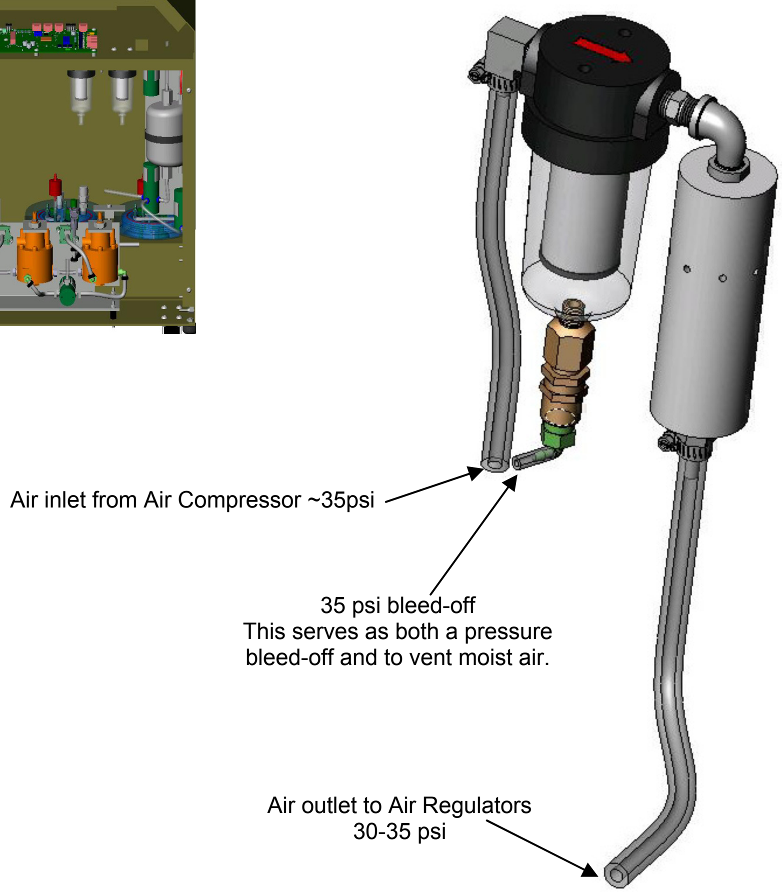
Error Condition	**Message 998: Waste subsystem halted due to lose of vacuum. If during operation, please check vacuum pump, waste quick connect or waste tank level.**
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)
Service Plug In	N/A

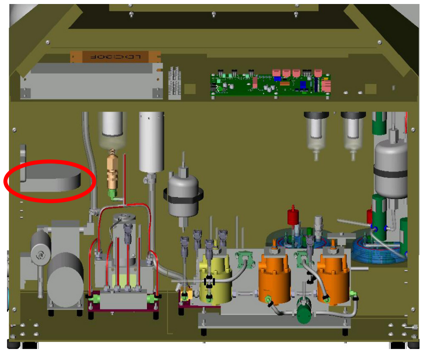
Error Condition	**Message 999: SMS Power fault. Reset SMS to continue. If this problem reoccurs, please call Dako for technical support.**
Send to Error Log?	Yes
Priority of Message (0-10)	1
Summit GUI Indicator Behavior	Message in top portion of CyAn Control Panel
Additional Notification in Summit	None
SMS – Summit Expected Behavior	SMS control – system shutdown
SMS Indicator Behavior	None
SMS State (Normal means anything but Standby – 0000)	1110 SMS int. error
Sheath Subsystem State	Non error state
Clean Subsystem State	Non error state
Waste Bit	Non error state
Sheath Load Cell 0 – 100%	N/A
Waste Load Cell 0 – 100%	N/A
Summit Operation Expected Behavior	Fluidics shutdown, CyAn idle
CyAn Pressure OK	OK
CyAn Vacuum OK	OK
621 Laser Fault	No fault (0)

Service Plug In	N/A



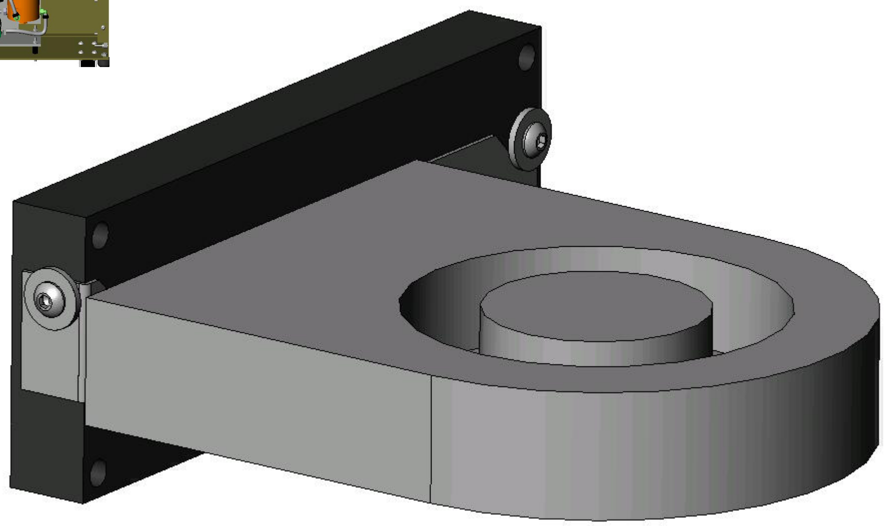
Air Dryer Assembly

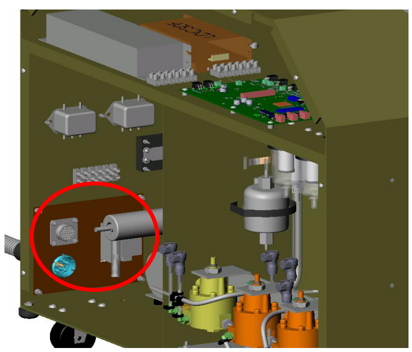




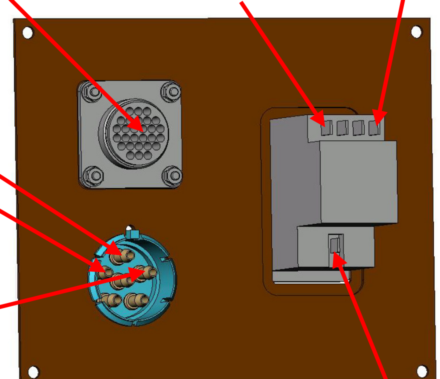
Turbine Fan Assembly

This fan creates airflow within the SMS to limit the effects of thermal changes to the system.

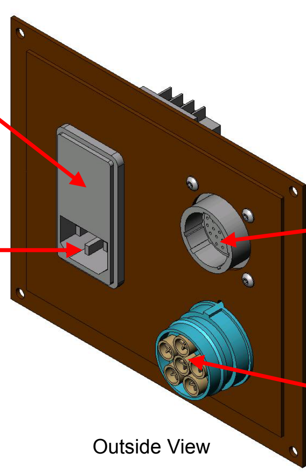


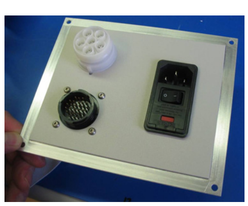


SMS Bulkhead Assembly

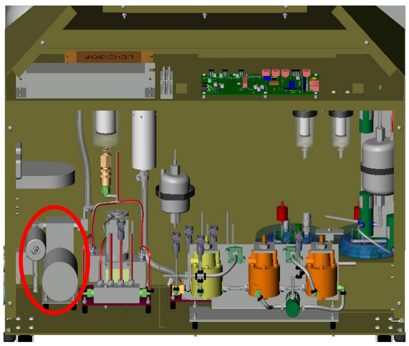


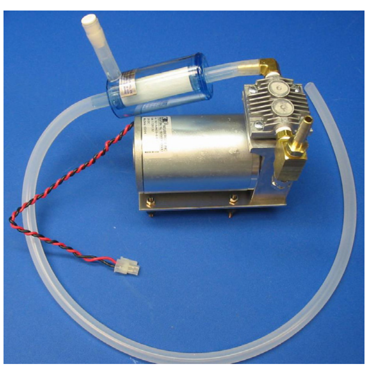
Inside View



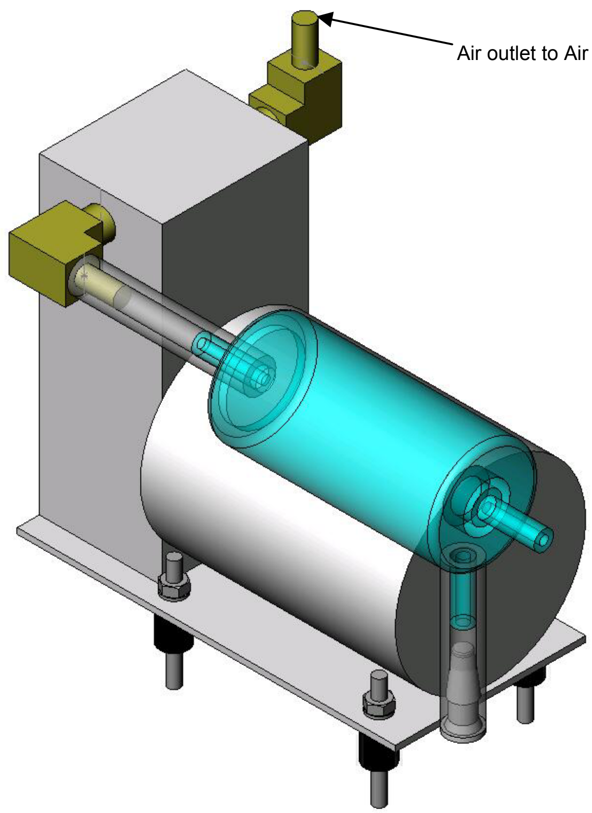


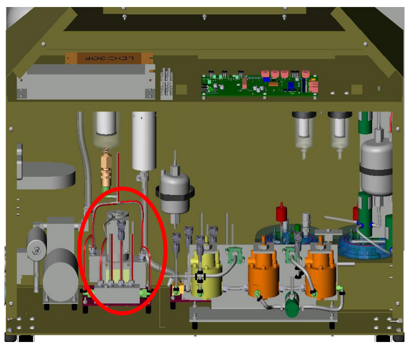
Outside View



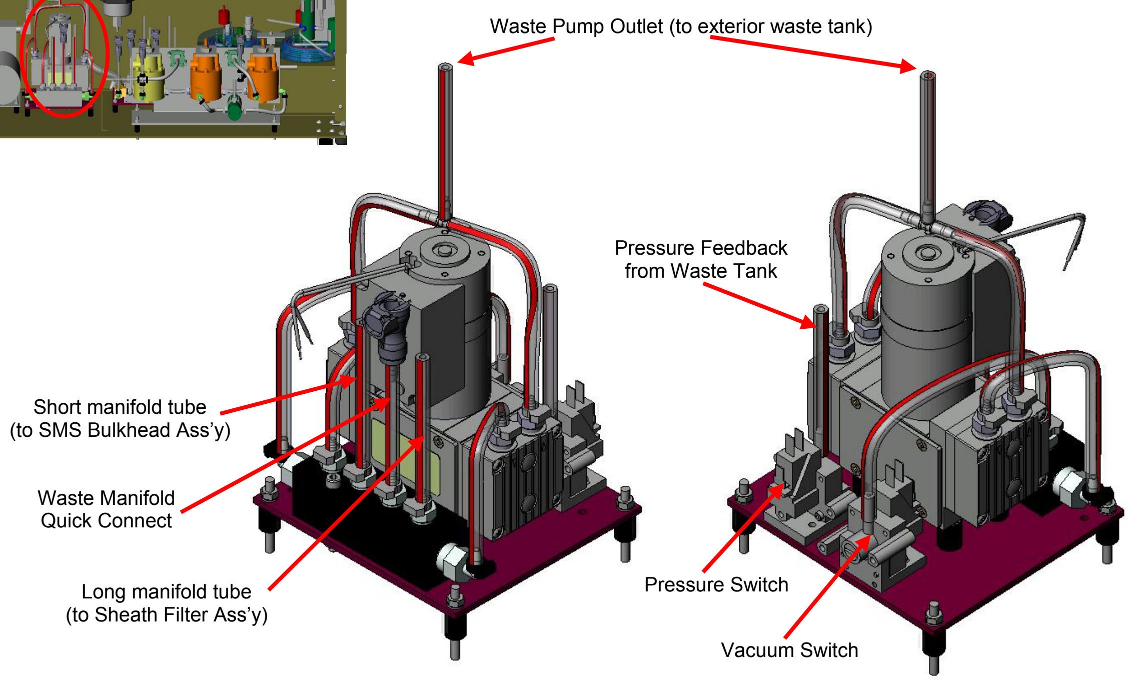


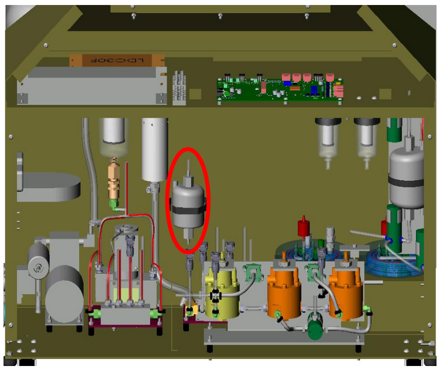
Air Compressor Assembly



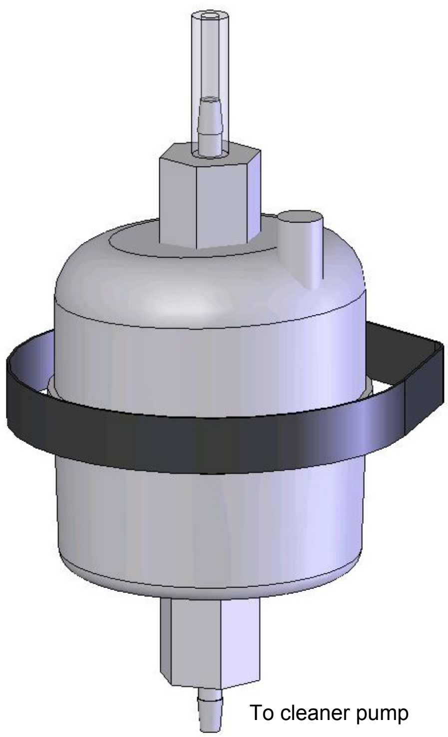


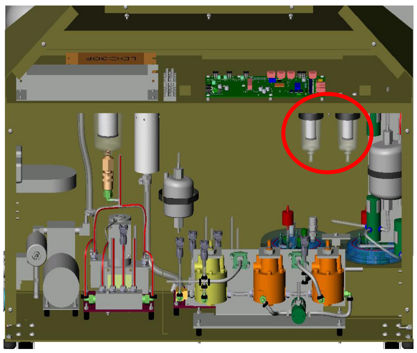
Waste Pump Assembly



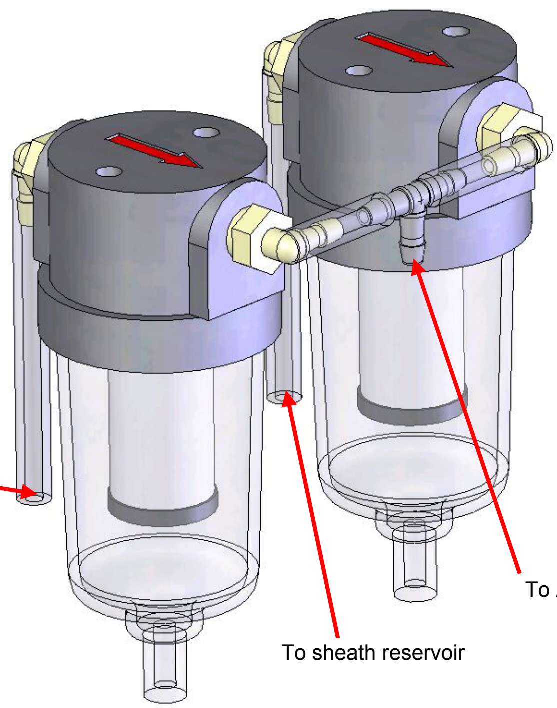


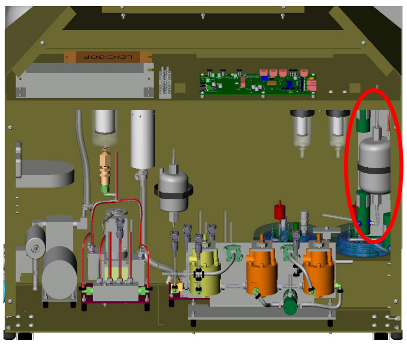
Cleaner Filter Assembly

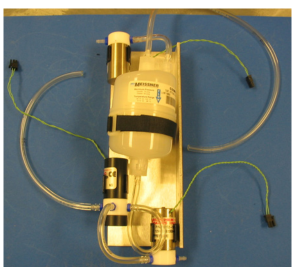




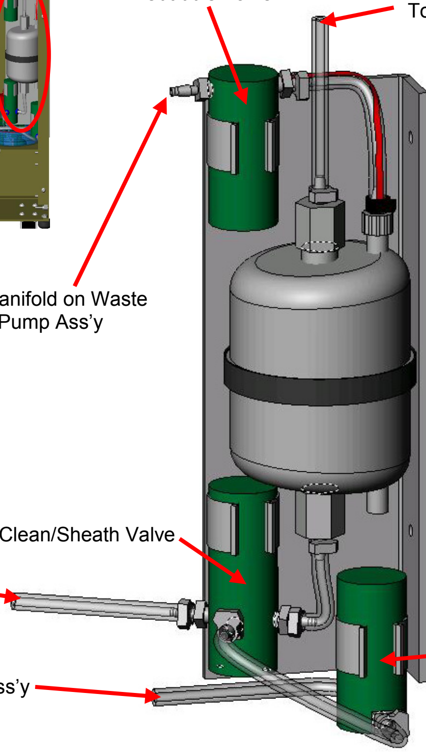
Coalescing Filters Assembly

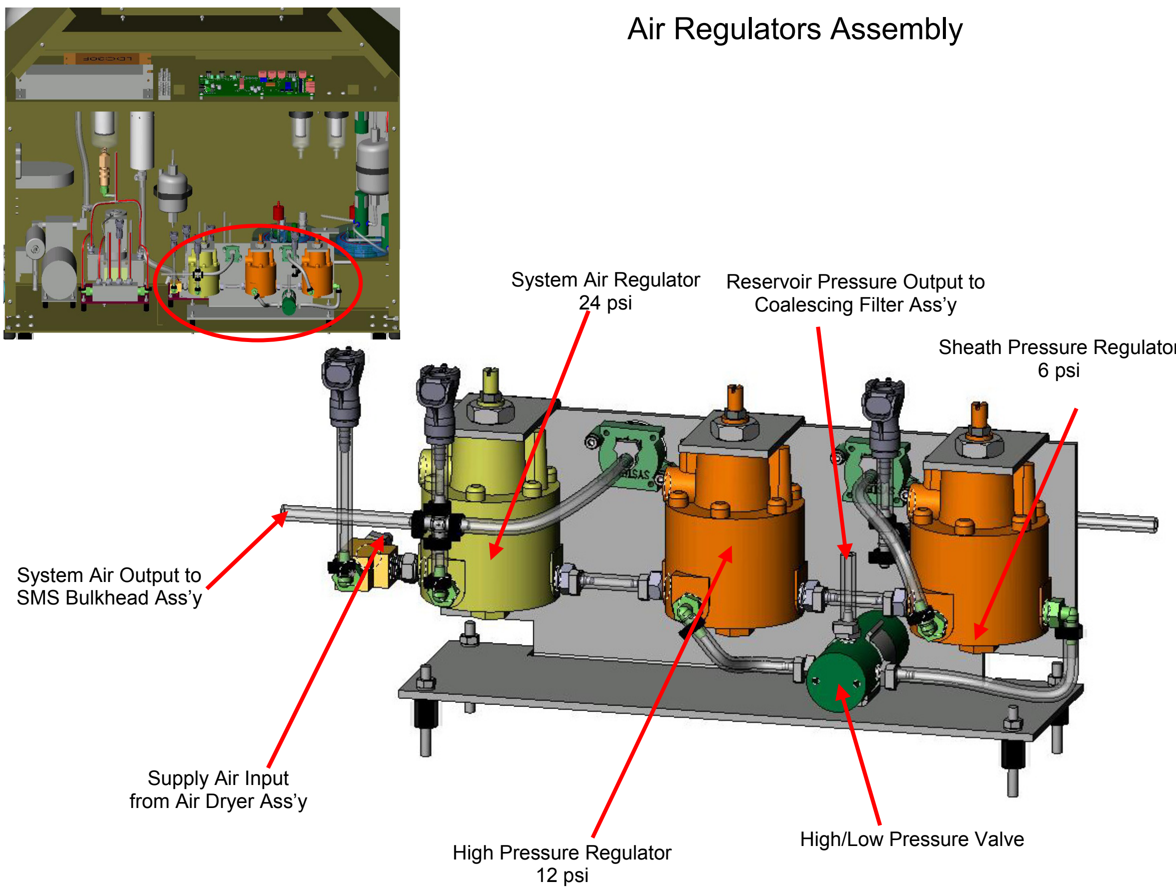


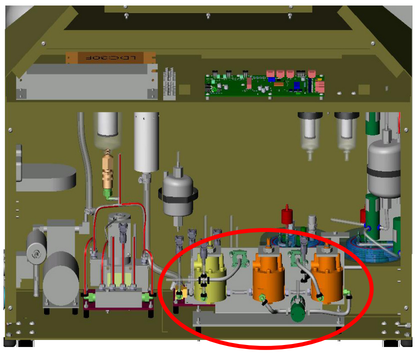




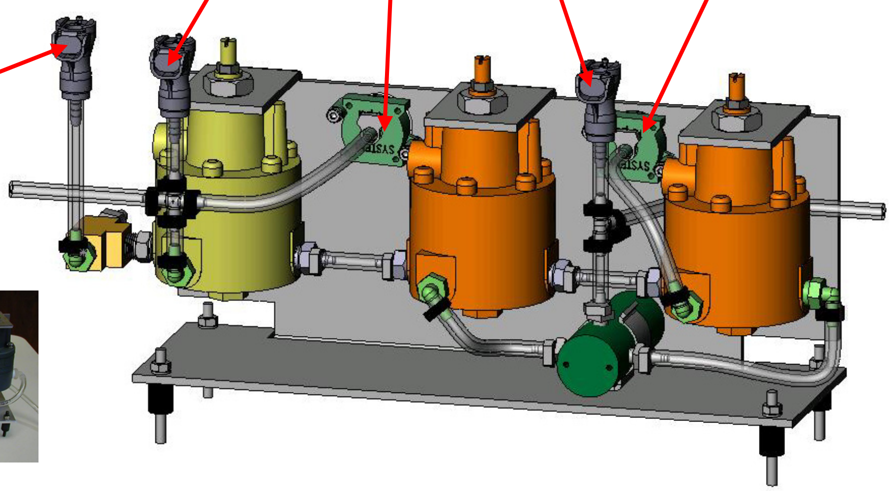
Sheath Filter Assembly

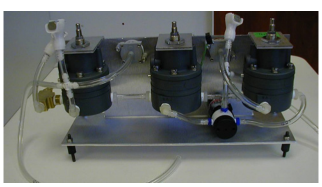


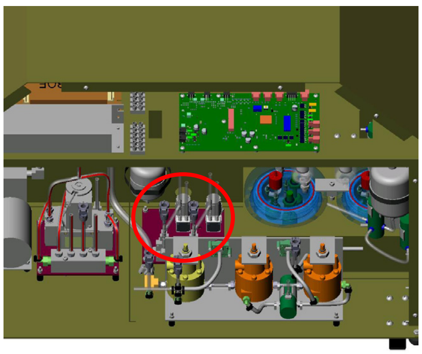

Air Regulators Assembly
System Air Regulator
24 psi
Reservoir Pressure Output to
Coalescing Filter Ass'y
Sheath Pressure Regulator
6 psi
System Air Output to
SMS Bulkhead Ass'y
Supply Air Input
from Air Dryer Ass'y
High Pressure Regulator
12 psi
High/Low Pressure Valve



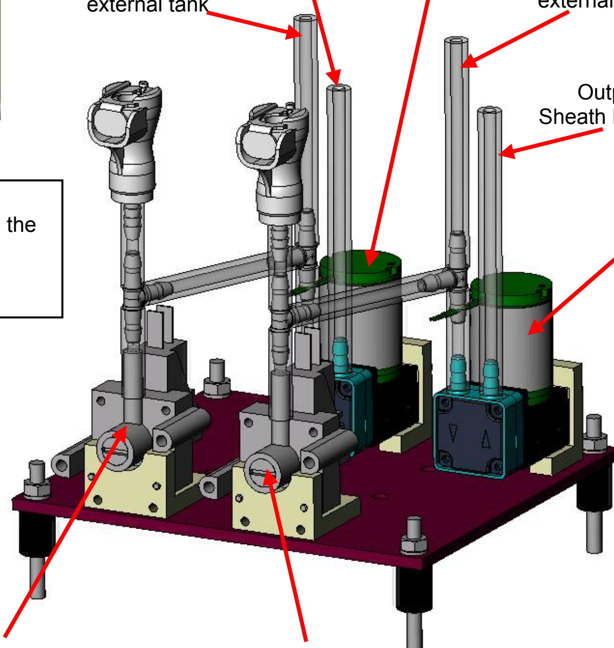
Air Regulators Assembly
(page 2)



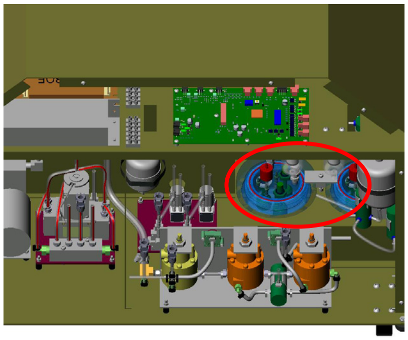




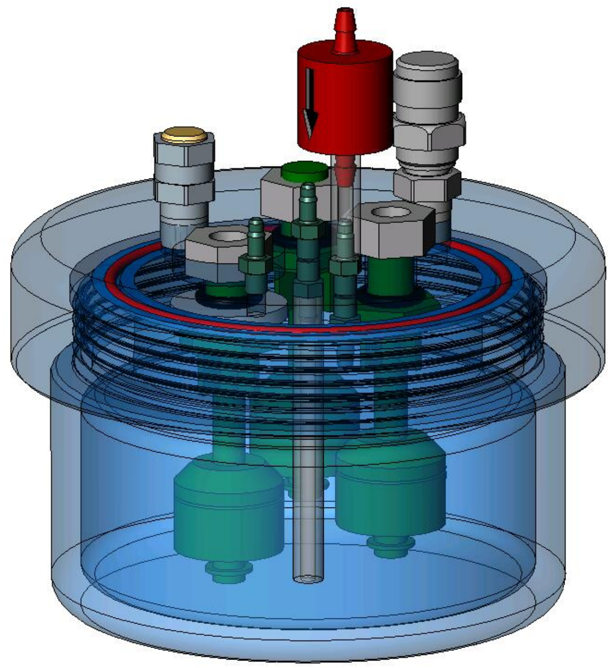
Sheath/Clean Pump Assembly



The liquid pumps run when the reservoir needs to be refilled. The pumps run while the middle float switch is closed (in the down position). The pumps run for only a few second at a time every 20-25 seconds.

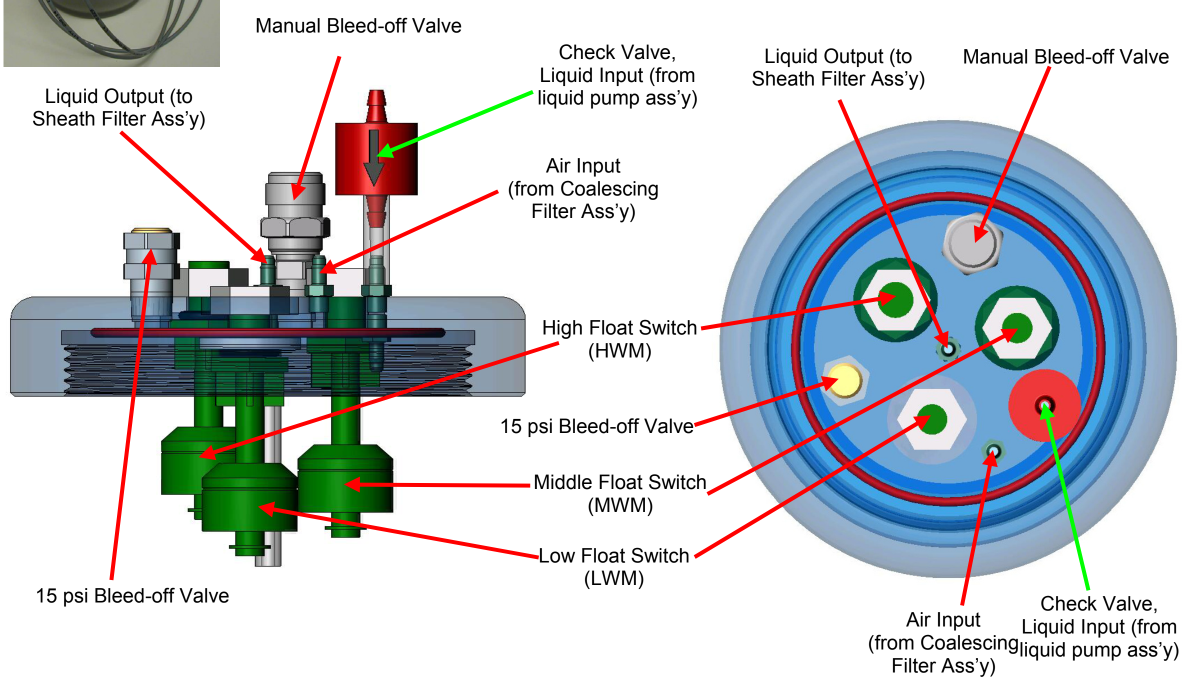


Sheath/Clean Reservoir Assembly

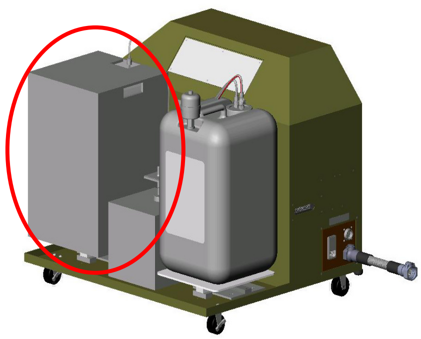


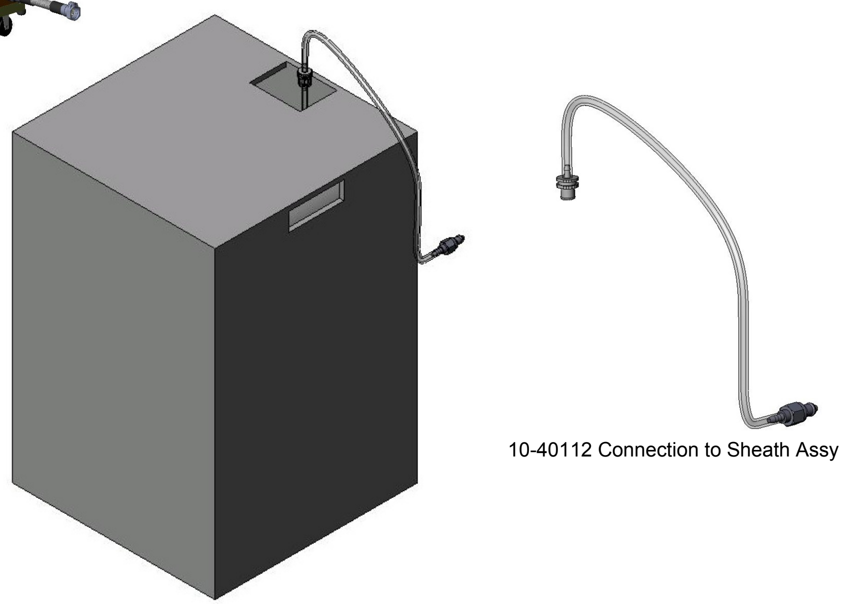
- The level of the reservoir is monitored by the three float switches. Under normal circumstances the liquid level is monitored by the histeresis of the middle float switch. This means that when the float switch is open (in the up position), the pumps are off. When the float switch is closed, the pumps turn on and replenish the reservoir. This makes for a cycle of the pumps being off for about 20-25 seconds and then on for about 3 seconds.
- Normally, the high float switch is closed. If the high float switch is open, the reservoir is overfilled.
- The low float switch will be open under normal circumstances. If the external container is empty and the pumps cannot refill the reservoirs, this float switch will eventually close, signifying that the reservoir is empty.

Sheath/Clean Reservoir Assembly (part 2)

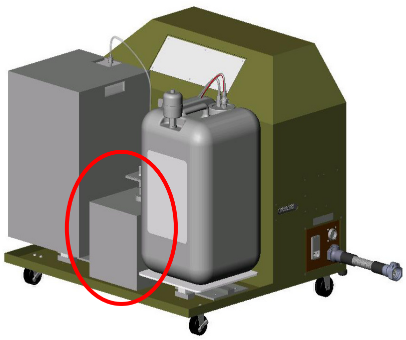


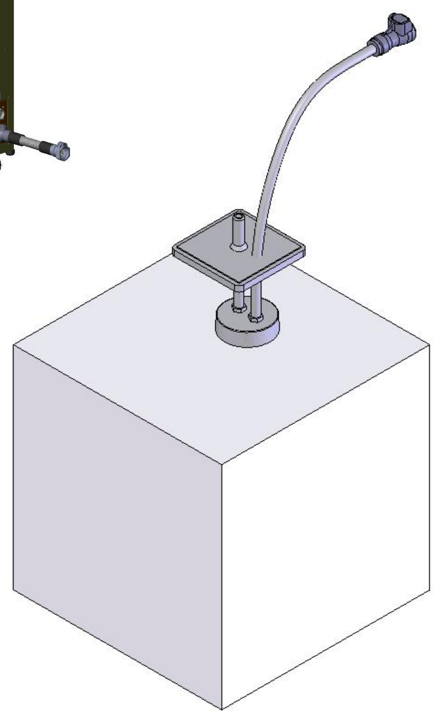
Sheath Cubitainer Assembly Connection to Sheath Assy

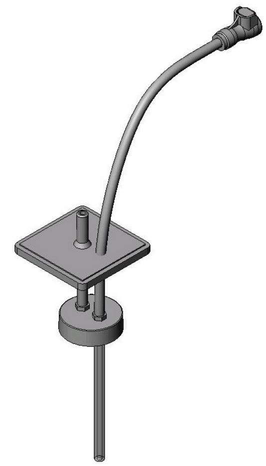




Cleaner Container Assembly
Cleaner Container Cap Assy

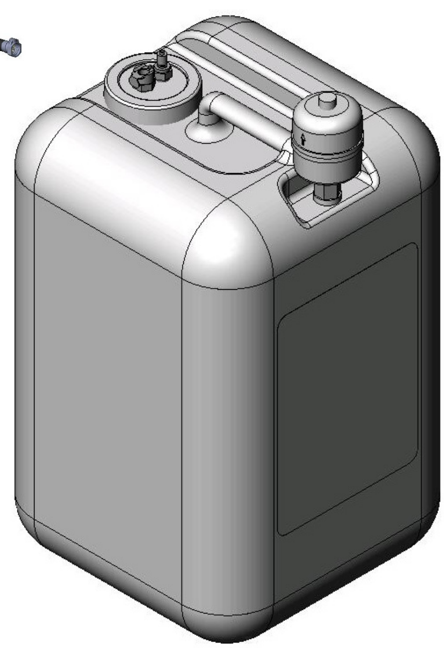


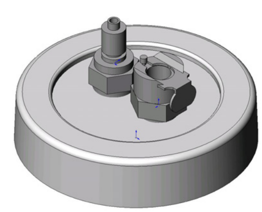




Cleaner Container Cap Ass’y

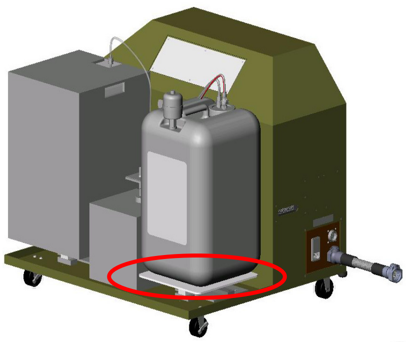
Waste Container Assembly
Waste Container Cap Ass’y
Connection to Waste Ass’y





Waste Container Cap Ass’y

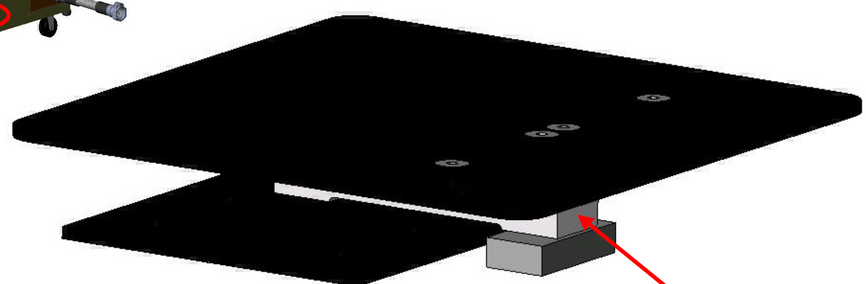
Connection to Waste Ass’y



Load Cell Assembly

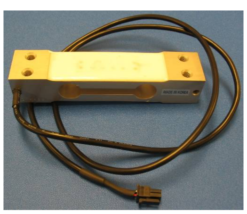
Spare, Load Cell: This only includes the load cell with its connector. It does not include the aluminum plates.

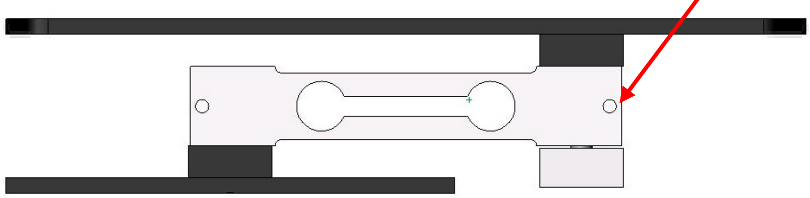
Refer to Doc # 0001080 for the Load Cell calibration procedure.



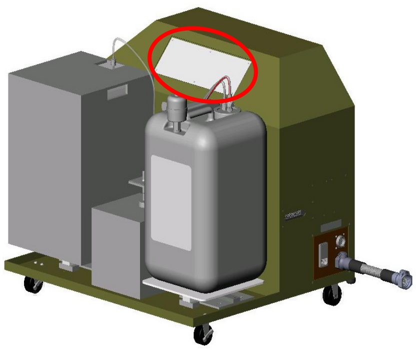
This is what measures the weight for the sheath and waste tanks.

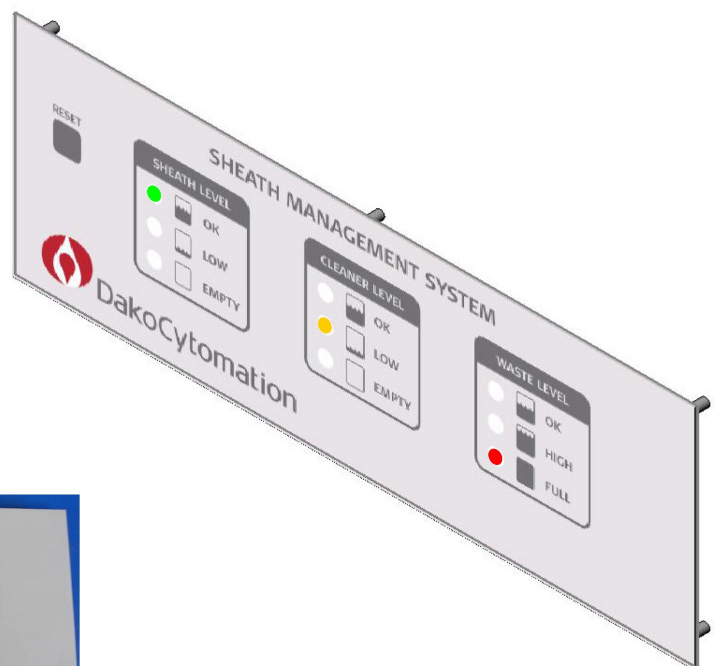
Load cell directional arrow should be facing down at this end of the load cell.

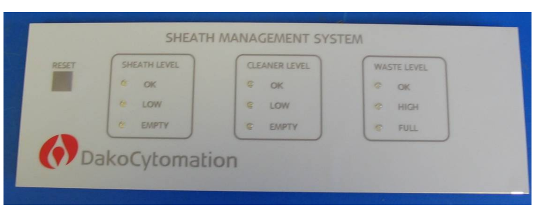


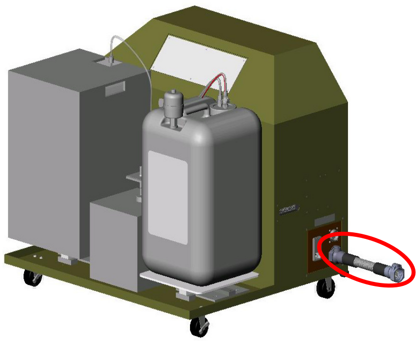


SMS Front Panel Assembly

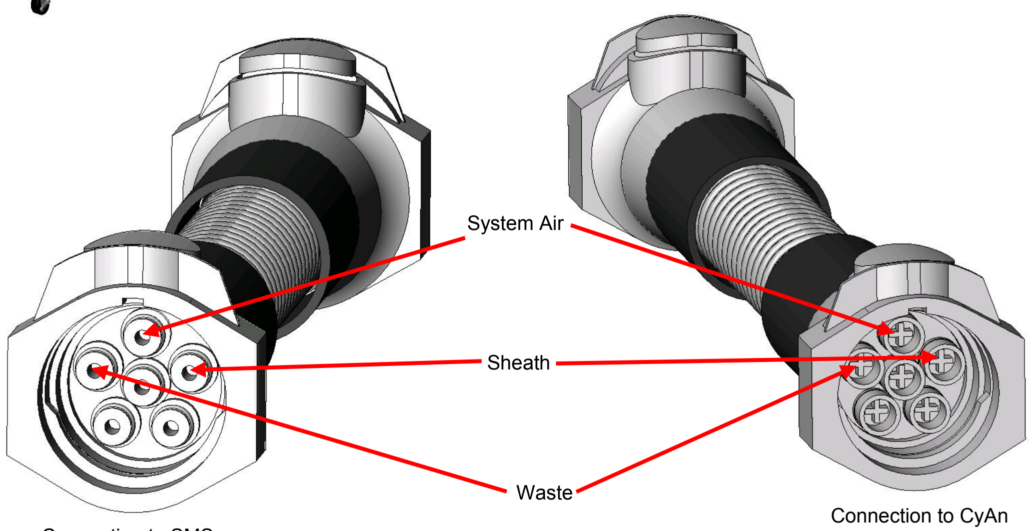




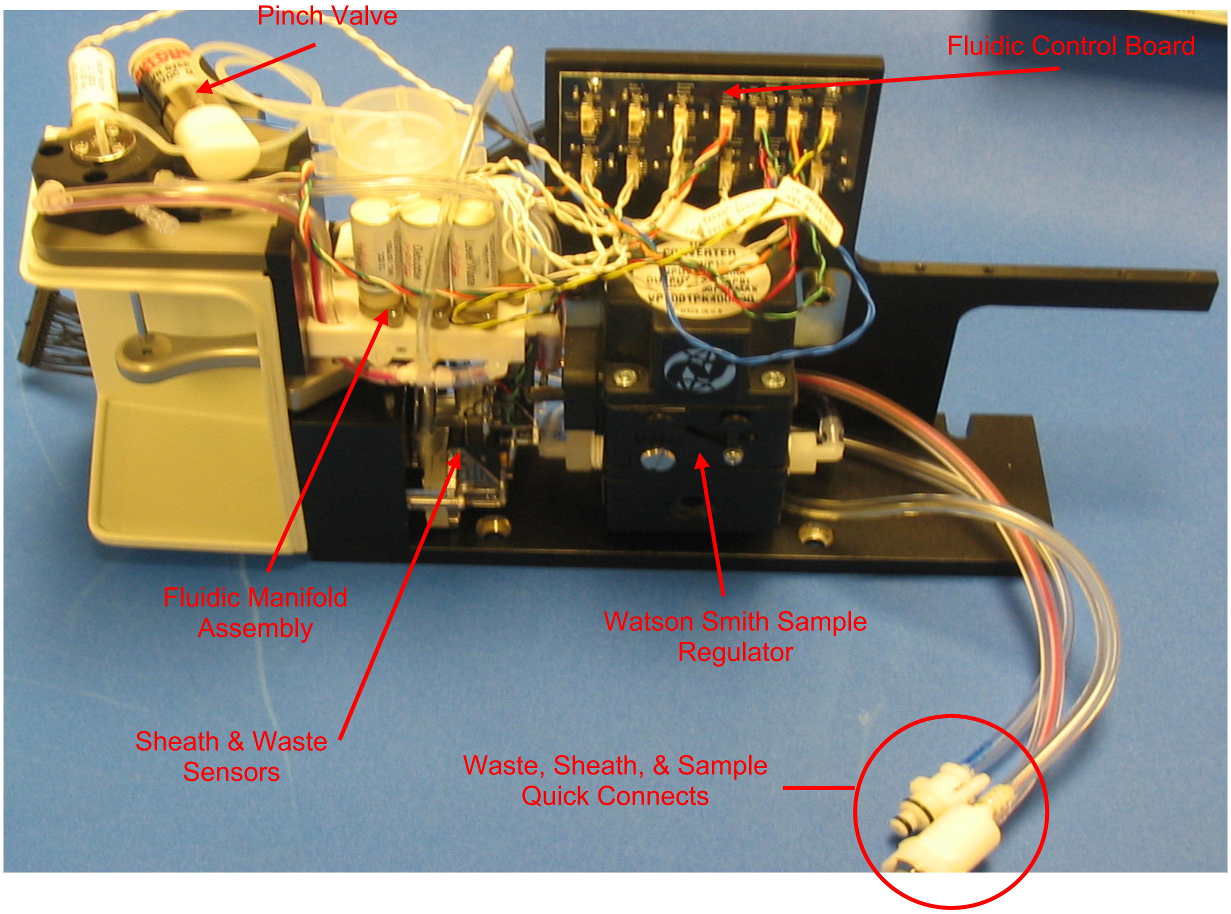




SMS Fluidics Hose



Fluidics Module



The fluidics module contains the sample pressure differential regulator (Watson-Smith), the fluidic valve manifold, fluidic control board, vacuum and pressure sensors, the sample uptake tube, and various tubing. The entire module may be separated from the CyAn ADP by removing two screws at the base of the module, disconnecting the quick connect fittings at the back of the module, and disconnecting the tubing going to the waste valve, sheath valve, debubble valve, and pinch valve.

Sample Uptake Stall

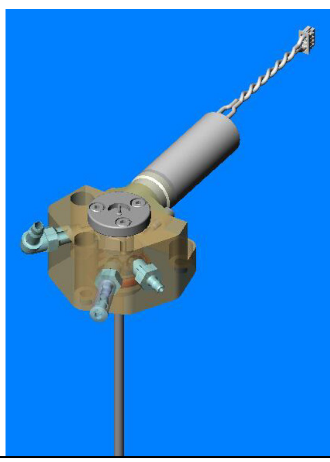
The sample uptake stall performs the following functions:

Backflushing

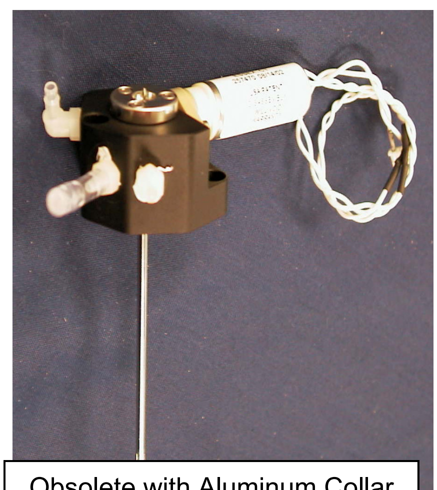
Backflushing is necessary to remove any residual sample from the previous sample before running the next. To backflush, with sample tube removed, move sample lever underneath sample uptake tube. If set up to do so in the preferences, the instrument will automatically backflush once the sample lever is moved into place. The backflush operation can also be activated by pressing the action button in the Control Panel while the sample lever is in the “in” position.

Sample Uptake
The sample will be aspirated when a tube is present and the sample lever is at the “in” position. The rate is determined by the Control Panel in Summit software. The percent number shown in the software is an arbitrary number that corresponds to a certain differential pressure. It is import to understand that by increasing the differential pressure too much will cause the core stream to become too wide which in turn will causes laminar flow to breakdown. This results in higher CVs and lower sensitivity. In order to increase the flow rate it is better to increase the concentration of the sample rather than increase the pressure differential too much. At this time, since the percent in the control panel is totally arbitrary it is not possible to state the maximum percent the sample should be run. Instead, a known concentration bead must be run to determine what is too high of a differential pressure. As a general rule, a concentration of one million particles per milliliter should run at a rate of 1000 events per second. So beads or cells at a concentration of 10,000,000 per ml should be able to run at 10,000 events per second.

Tube in Tube Assembly



Current with Ultem Collar



Obsolete with Aluminum Collar

The current design of the tube in tube has an ultem (a plastic) collar. This collar is less likely to precipitate salts out of solution when compared to the obsolete style of the aluminum collar. If a unit has the aluminum collar style, it should be replaced with the ultem style assembly.

Flow Cell- ADP without O2 Optics

The flow cell is the heart of the instrument. It is made of an upper and lower body. The rotational position of the flow cell to the laser beam is very important. When replacing the flow cell without O2 Optics, it is important that the back reflection of the 488 laser beam should go directly back down the 488 beam path. In this way, a perpendicular 90 degree angle of the beam to the flow cell is achieved.

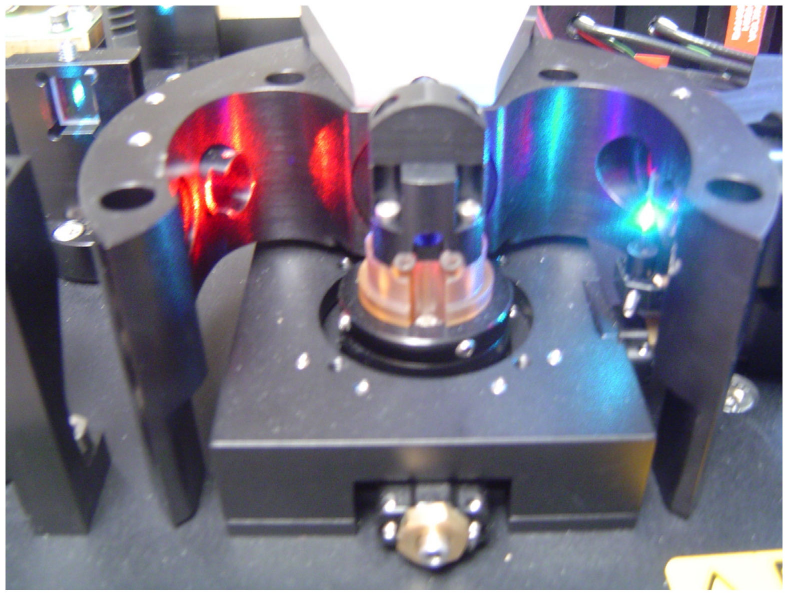
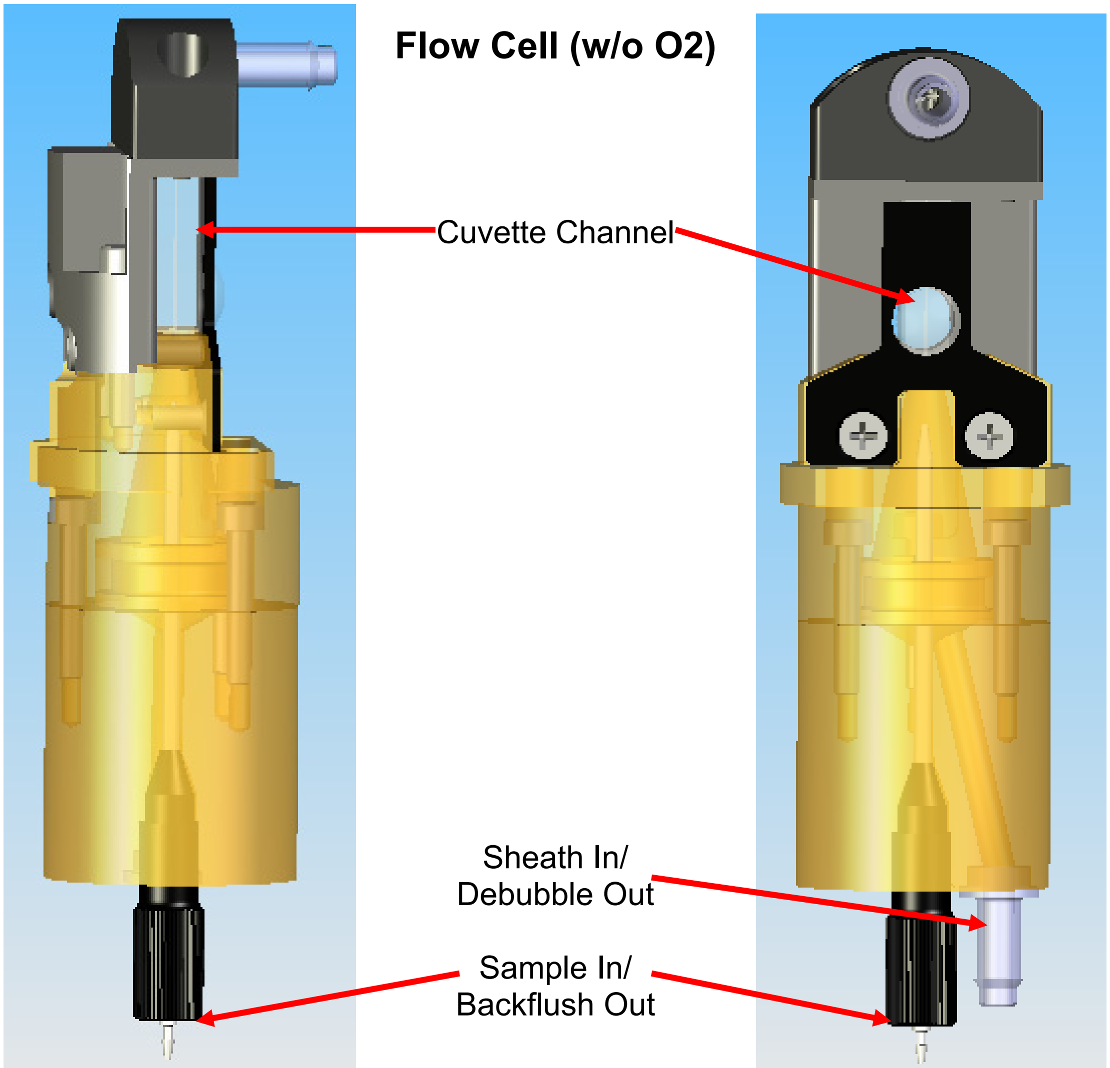
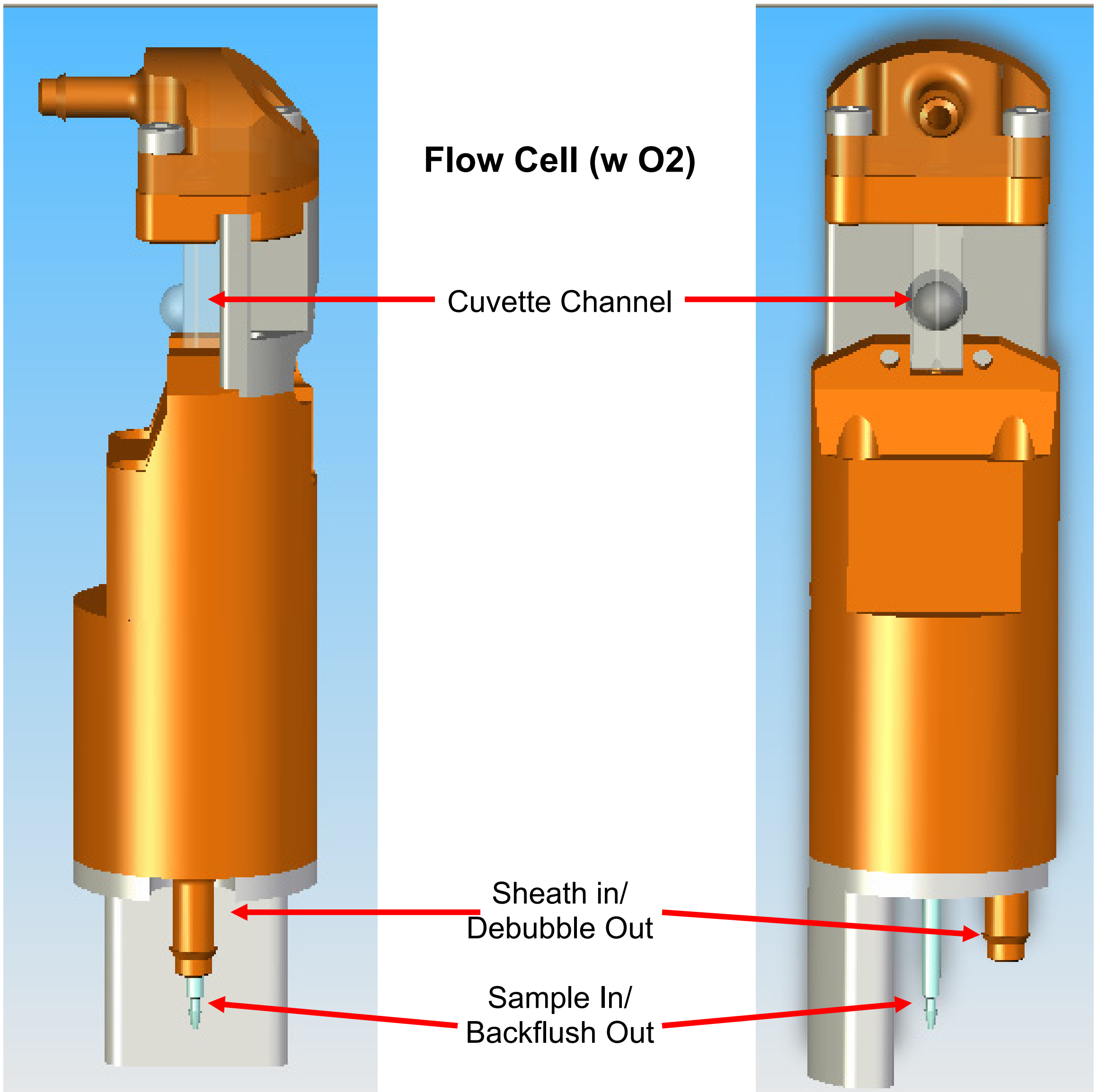
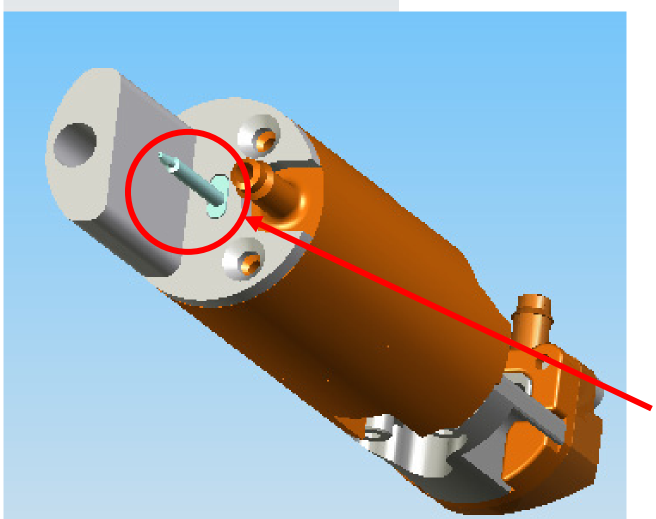


Figure 3.5: Flow Cell Assembly without O2 Optics (left) and flow cell location in CyAn ADP without O2 Optics (right).


Flow Cell (w/o O2)
Cuvette Channel
Sheath In/
Debubble Out
Sample In/
Backflush Out

Flow Cell- ADP with O2 Optics





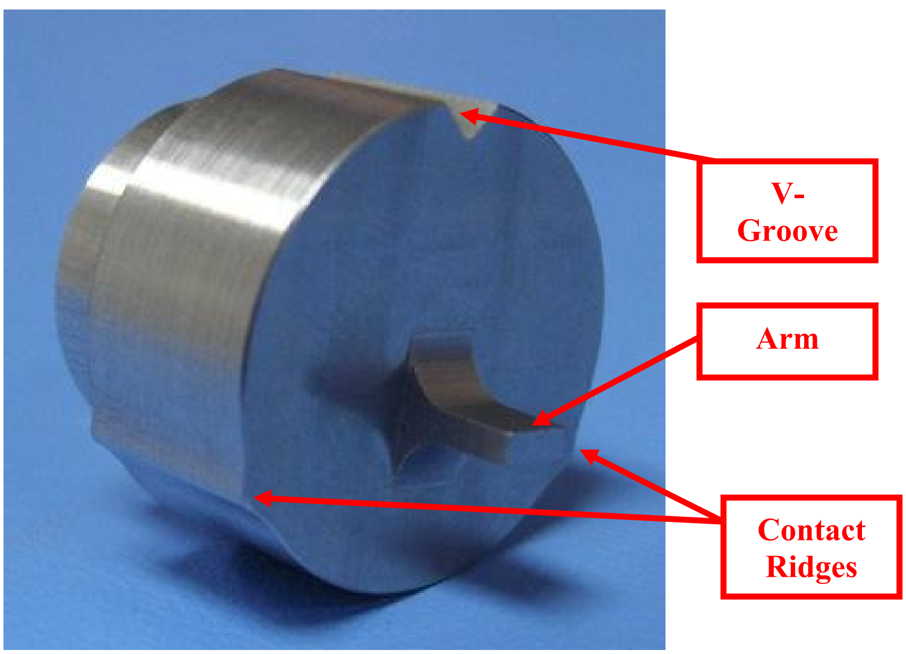
The sample needle for the flow cell with O2 has a preset height and orientation. If replaced, it should only fit into the flow cell in its proper orientation and height.

Theory of Operation of Flow Cell

Sample flows though a channel within the cuvette. While in the cuvette, fluorescence molecules attached to the sample are excited by laser light and emit light signal. The cuvette with lens combination is the first optical element in the collection optics used to image the excited sample/object. Because light is emitting in all directions, the lens on the cuvette is used to gather as much of this light as possible and decrease the divergence.

O2 Flow Cell Tool

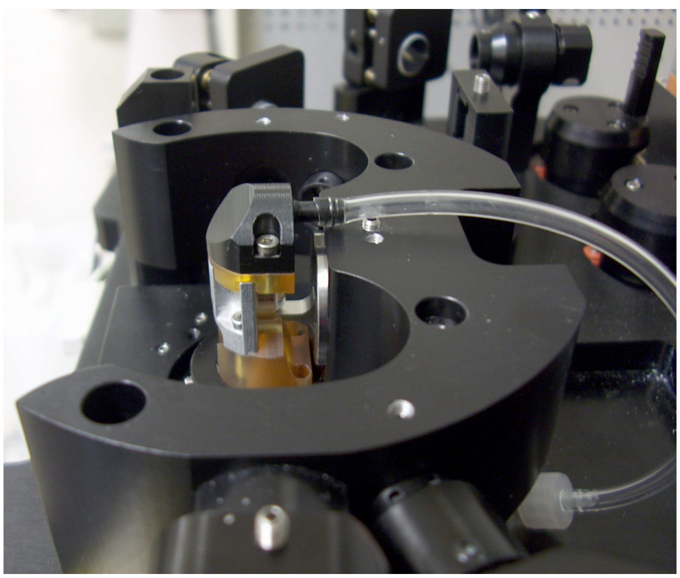
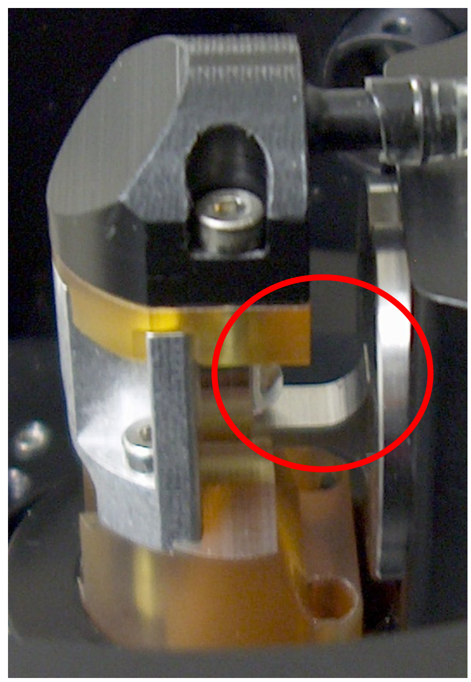
The flow cell for the O2 Optics has its position set by the flow cell adjustment tool. This tool is placed within the “claw” where the objective would normally reside. The arm on the tool is positioned so the optic on the flow cell is just above it and the cuvette is flat against it. This will set both the height and the rotation of the flow cell. When the position is correct, the two set screws in the collar of the x-y adjustment stage should be tightened. See procedure below for full instruction.



Flow alignment tool for use with the O2 Optics.

O2 Optics Flow Cell Alignment Procedure:

1. Insert O2 flow cell into the flow cell stage. Do **not** fully tighten stage set screws. (Note: there is no lip on the flow cell to adjust the height.)
2. Use the Flow Cell alignment tool to adjust the height and rotation of the flow cell.
    a. Insert tool into space where the objective normally resides (from the back) with the arm towards the flow cell. Place one of the cone point set screws (provided with the tool) into the center tapped hole where the objective set screw would normally reside.
    b. Orient the tool such that the cone point set screw is aligned with the v-groove on the tool and tighten the tool into place. The arm of the tool should extend out far enough to contact the flat cuvette surface below the lens attached to the cuvette.
    c. Ensure the cuvette is square to the tool and the lens is resting on the tool.

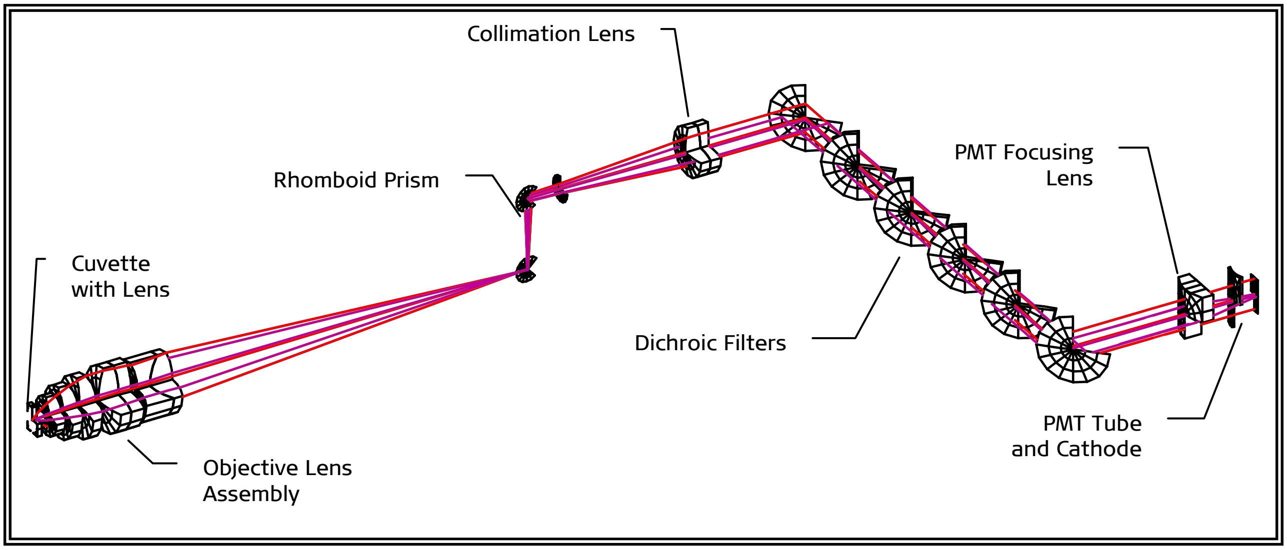



Flow cell height and rotation alignment tool (note: this flow cell is a prototype, silver piece will be finished black).

Holding the flow cell assembly in place against the tool, tighten screws to fix the flow cell height and rotation. (Note: it is better to tighten each screw a little at a time and go back and forth between then instead of completely locking in one and then the other)

d. Back off the flow cell from the claw completely and remove the cuvette alignment tool.
e. Connect sample and sheath input tubes to the bottom of the flow cell.

Optical Table



Collection Optics Optical Element Layout

The optical table is where the PMTs (photomultiplier tubes), diode lasers, beam-shaping optics, steering mirrors, flow cell, pinhole camera, and forward scatter detector are located.

Fig 3.6: CyAn ADP Optical Table
with out O2 Optics

1. Violet (405) Diode Laser
2. Blue (488) Diode Laser
3. PMTs and Optical Filters
4. Violet Diode BSO Assy
5. Collimating Lens Block with Pinhole Strip
6. Red (635) Diode Laser in adjustment stage
7. FSC Detector

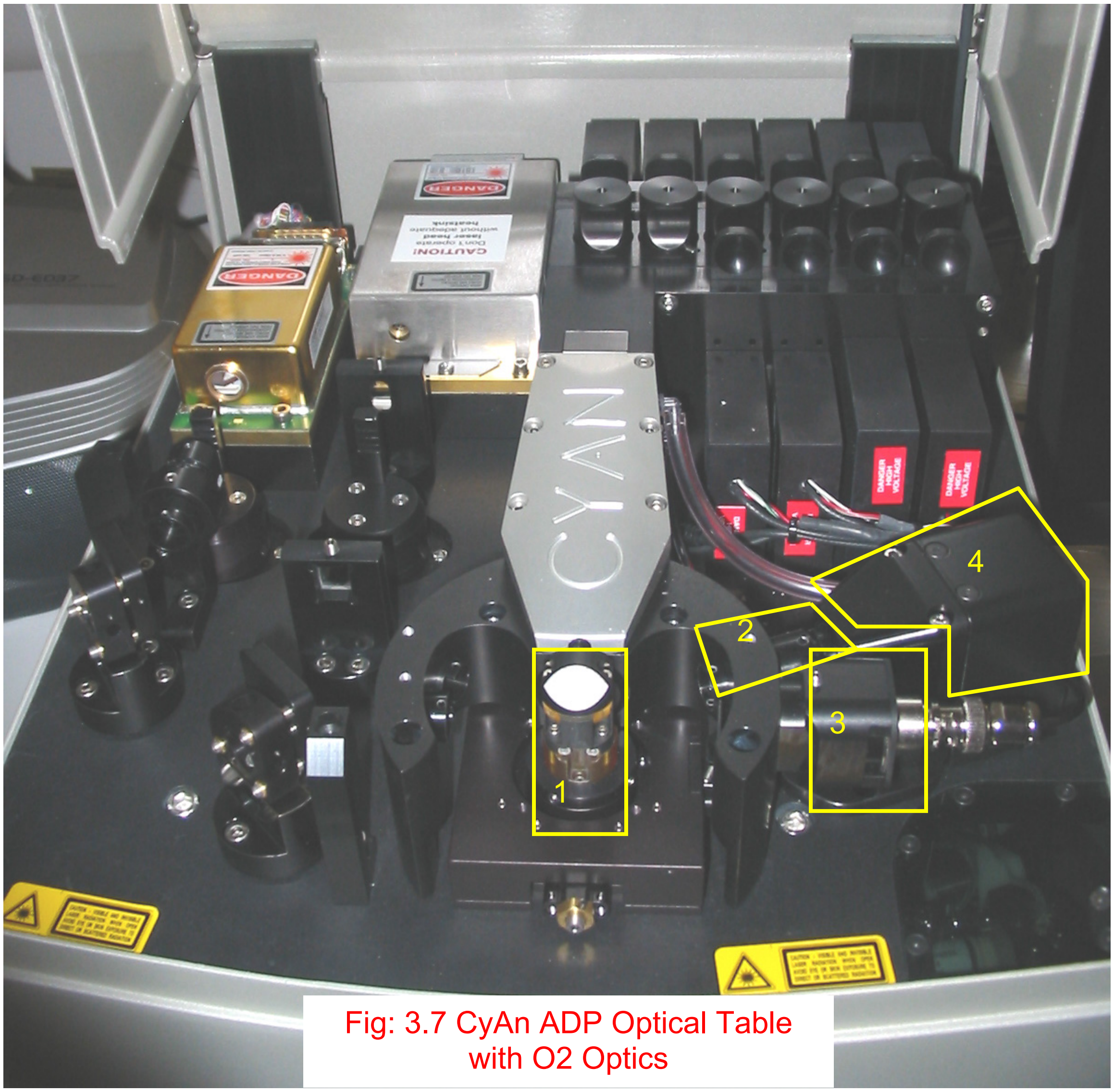
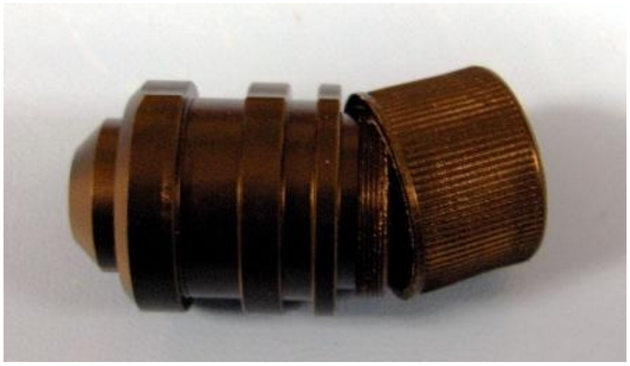


Fig: 3.7 CyAn ADP Optical Table
with O2 Optics

1. O2 Optics Flow Cell
2. Red Diode Beam Shaping Assembly
3. Forward Scatter Holder

   The O2 Optics FSC Holder will work on older model CyAns, however the older model CyAn FSC Holder will not work with the O2 Optics.
4. Red Diode Cover

O2 Optics Objective Assy



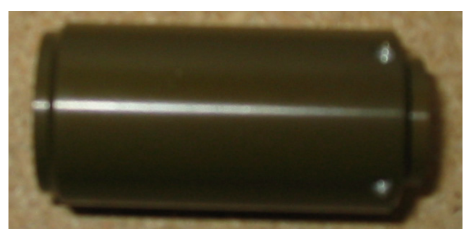
Theory of Operation of Objective lens assembly

The objective lens assembly has three main functions, to collimate collected light, correct for chromatic aberrations incurred and to create a focused image of the object. The first three crescent shaped lenses are used to collimate the divergent light coming from the cuvette lens. Following the meniscus lenses are two achromatic doublets. These lenses are used to correct chromatic aberrations incurred in the first few lenses of the design. The second function of the achromatic doublet is to focus the collected light onto the pinhole strip.

Procedure for install O2 Optics Objective.

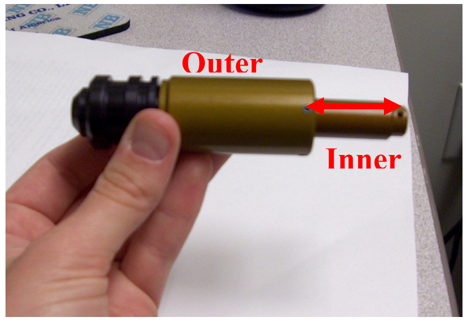
1. Install the objective using the objective alignment tool

Alignment tool in extended position



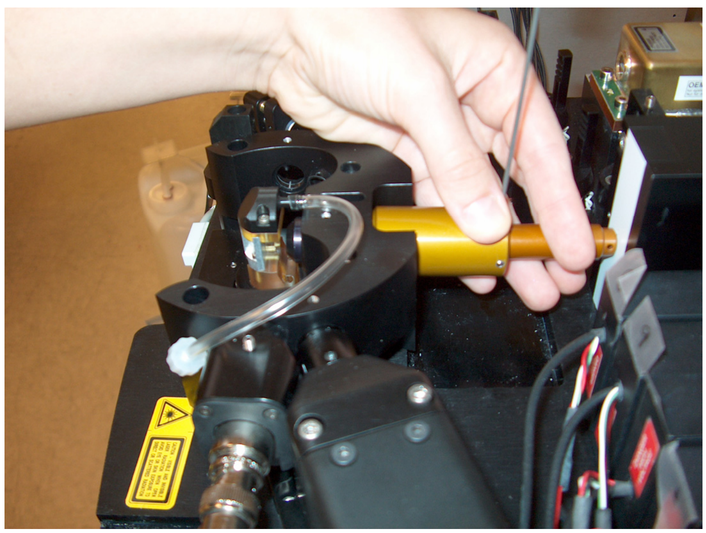
Alignment tool in compact position

1. Attach the O2 Optics objective to the objective alignment tool. Make sure it is **not** in the extended position.



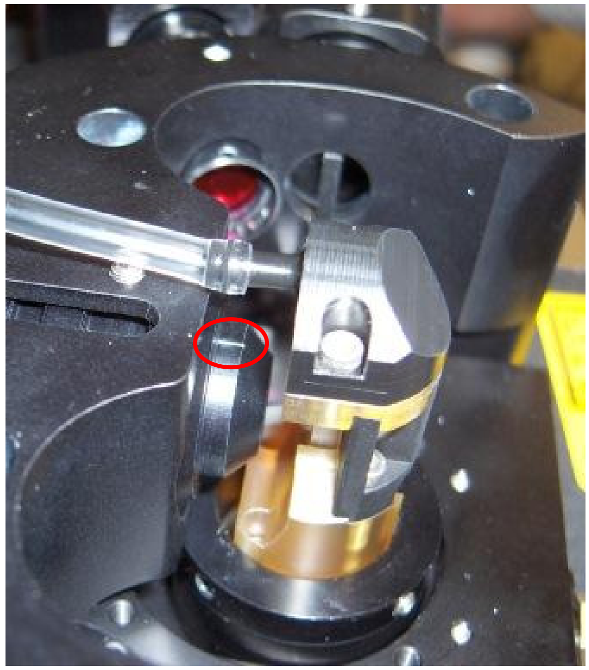
Objective alignment tool with objective attached.

2. Insert the objective into the claw.
3. Holding the inner tube of the tool against the pinhole strip, extend the tool fully and lock it into position with 1.5 ball driver. Use caution to make sure the front of the objective **DOES NOT BUMP** the flow cell.



Fully extend alignment tool and tighten set screw to fix the length

4. Making sure the objective contact points are facing down and the white mark is facing up, hold the end of the alignment tool flat against the pinhole strip and lock the objective into place. (Note: tighten the set screw slowly and make sure it is not tilted.)



White mark on the objective should be facing up to ensure three contacts.

5. Loosen the screws on the tool to retract the position. Unscrew the tool from the objective. The objective position is now set.

Detection Block

The detection block is made up of five main parts.

Pinhole Strip

The pinhole strip was designed to separate the different light paths spatially. Since there can be three laser lines used to simultaneously excite particles there are three different signals to be collected. The pinhole strip increases the physical space between these paths in order to properly detect the three signals separately without crosstalk while maintaining light travel parallel to the optics mounting plate.

Culminating Lens Block

In each path there are one or two elements used to collimate the three light paths after being spatially separated. This is a way to maintain the signal through the remainder of the system. Placement and use of specific achromatic lenses are used in order to maintain the color quality of the system.

Dichroic Block and Dichroic Filters

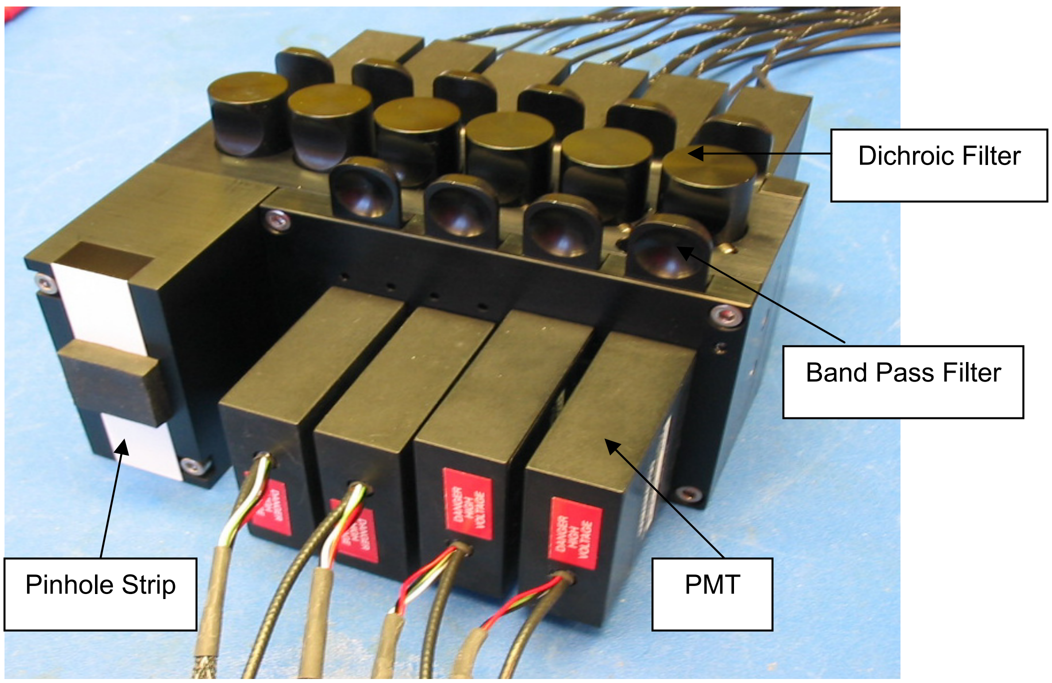
Dichroic filters are used in each path to optically separate multiple signals contained within a single light path. Next, band pass filters are used to further separate out the desired signal bound for a single PMT.

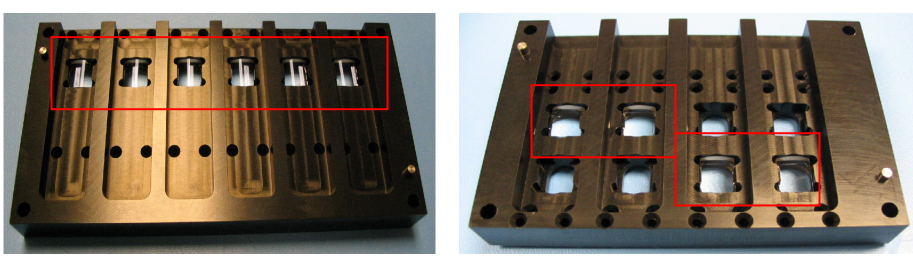
PMT Plates with Optics

A single cylindrical lens is used directly after the NI filters. This lens is used to form the signal to match the shape of the active area of the PMT.

PMTs

The PMT is the final element in the optical path and is used to detect photon signal (light) and convert it into an electronic signal.





PMT plates for O2 Optics. The boxes show the optics that focus the light onto the PMT.

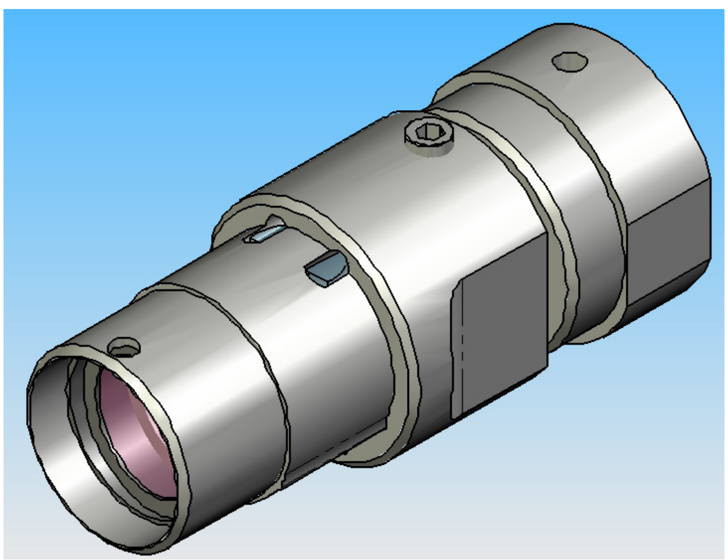
Miscellaneous Parts Located on Optical Table

Path 1 & 3 Shutter

Path 2 Shutter w/o O2 Optics

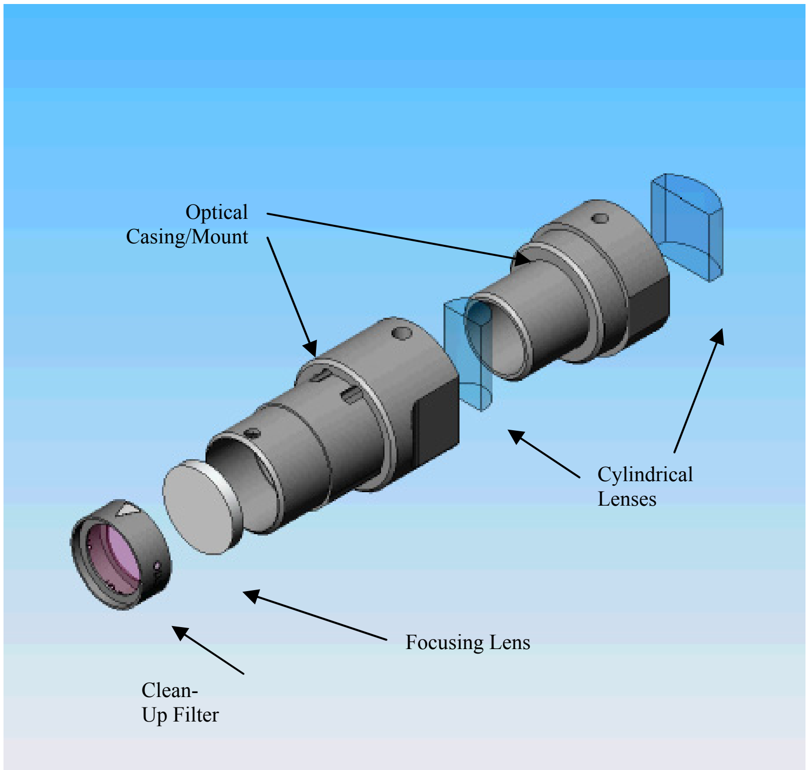
Pinhole Camera Assy

Waste Reservoir Assy



Red Diode Beam Shaping Assembly (O2 Optics Only)

The Beam Shaping Assembly is made up of optical components mounted within a casing. It is designed to optically shape the red diode laser beam prior to focusing down onto the inspection point at the stream. The resulting beam at the inspection spot is elliptical in shape to provide a larger more uniform excitation spot allowing for greater alignment stability. In addition, the Beam Shaping Assembly purifies the spectral output of the red diode laser, thereby reducing the optical noise introduced from the laser.



Specific Functions of Red Diode Beam Shaping

(Note: This assy cannot and should not be taken apart in the field. If needed, a new one should be ordered)

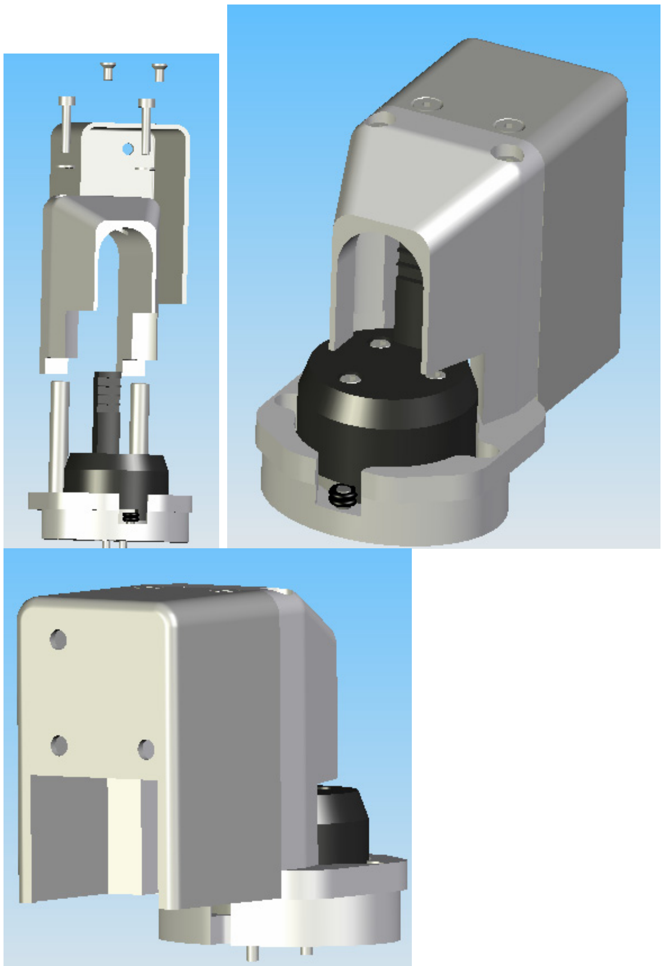
Cylindrical lenses – The cylindrical lenses used in the Beam Shaping Assembly are used to condense the horizontal axis of the Gaussian laser beam to produce an elliptical spot size of the correct proportion.

Focusing lens – The focusing lens is used to create a minimum beam size at the point of inspection.

Clean-up Filter – The clean-up filter is a band-pass filter that transmits a narrow wavelength band (in comparison to possible band size emitted by the RDL). This allows for excitation and scatter to be from a defined, narrow wavelength.

Optical casing/mount – This mechanical part is used to mount the above described optics and provides adjustment to align the cylindrical optics held. It is designed to be held within the “claw” and has built in features to allow for focus adjustment when aligning the CyAn instrument.

Diagram of Path 2 Shutter (O2 Optics Only)



CyAn Control Board

This board controls almost every aspect of the CyAn. It is the center point for information coming to and from the CyAn. The following information describes the main inputs and outputs of this central board.

Power supply monitoring:

On the CyAn (non UV) there are three power supplies that are monitored on the control board. These are the +5V (from the PXI power supply), 12V Auxiliary (behind control board), and the 12V laser power supplies (rear of the instrument).

On the CyAn w/ UV there are only the +5V and 12 V Auxiliary power supplies are monitored. (The laser power supply is Enterprise II power supply which is located externally to the CyAn.)

Power Supply Fusing and Filtering:
The control board has resettable fuses to protect it and those items connected to the control board. The voltages coming into the control board are as follows +12V, -12V, +5V (these three come from PXI power supply) and Aux 12V (from power supply behind control board). In addition, the CPLD uses3.3V which is generated from this input from the 5V power supply. There are two separate filter circuits that are used to draw off of the board. One is for the PMT circuitry and one for the rest of the control board. These filter any noise the power supplies might generate.

CPLD Basics:

This has been called the “Watch Dog” in the past, however it performs much more than this. It allows for the CyAn to operate independently of the software. With out this the CyAn would shut down anytime Summit was closed. It is also where are all command instructions from Summit are initially routed before they reach other locations on the board and the CyAn. Last, it performs error corrections if incomplete instructions are received from Summit software by requesting Summit to resubmit its commands.

The Watchdog feature is expressed through the following four states:

Halt: When told to shutdown by the user or if power supply voltages dip below acceptable levels (90% of specified voltage) the control board will enter this state. When in this state, all fluidics and all optics will be turned off. During this state, summit no longer controls the instrument.

Run: This state is when the system is running normally and the software is communicating properly to the CyAn. In this state Summit has full control of the instrument.

Safe: If communication from Summit is lost then the board will go to this state. This occurs when Summit is improperly shutdown or crashes. This will cause the sample pinch valve to close to prevent sample from running, the sheath valve to close to prevent sheath from running,

keep some waste valves open so no waste can back up, and leave the lasers on while closing the shutters. In order to come out of Safe state the software must reinitialized the CPLD. If this cannot be done (due to computer failure) then the Cyan must be physically powered down or the reset button in the LED cover board must be pressed using a small pointed object.

Shutdown: This is the warning state for summit before going into safe state. During this state, summit still has full control of the instrument.

Optics Control:
As long as the lasers are on and the shutters are open, the LED indicators on the top cover will be illuminated. If either the laser power is off or the shutter is closed then the LED indicator will be off.

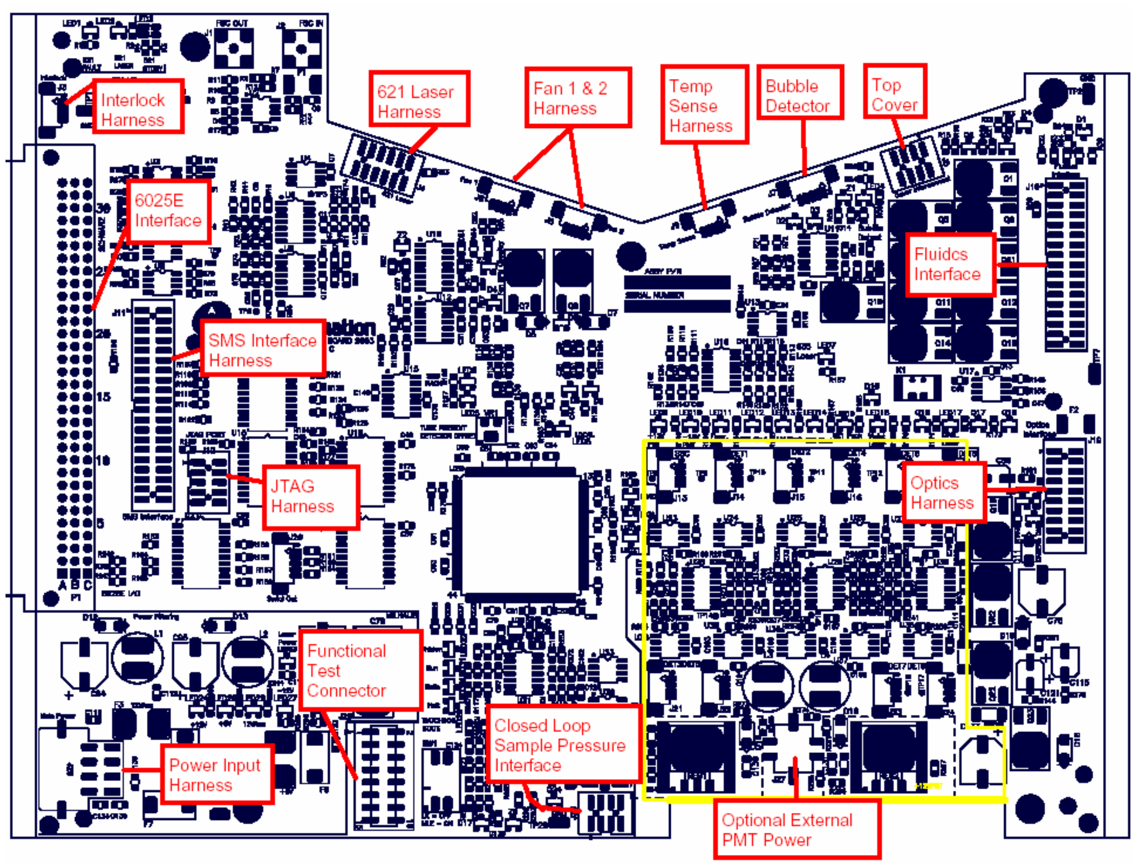
There is a 621 laser peeking circuit on the control board. This can be utilized by using the I/O test software.

The PMT voltage is dictated by Summit through the control board. The inputs lay on their own plane of the board and are shielded from noise production.

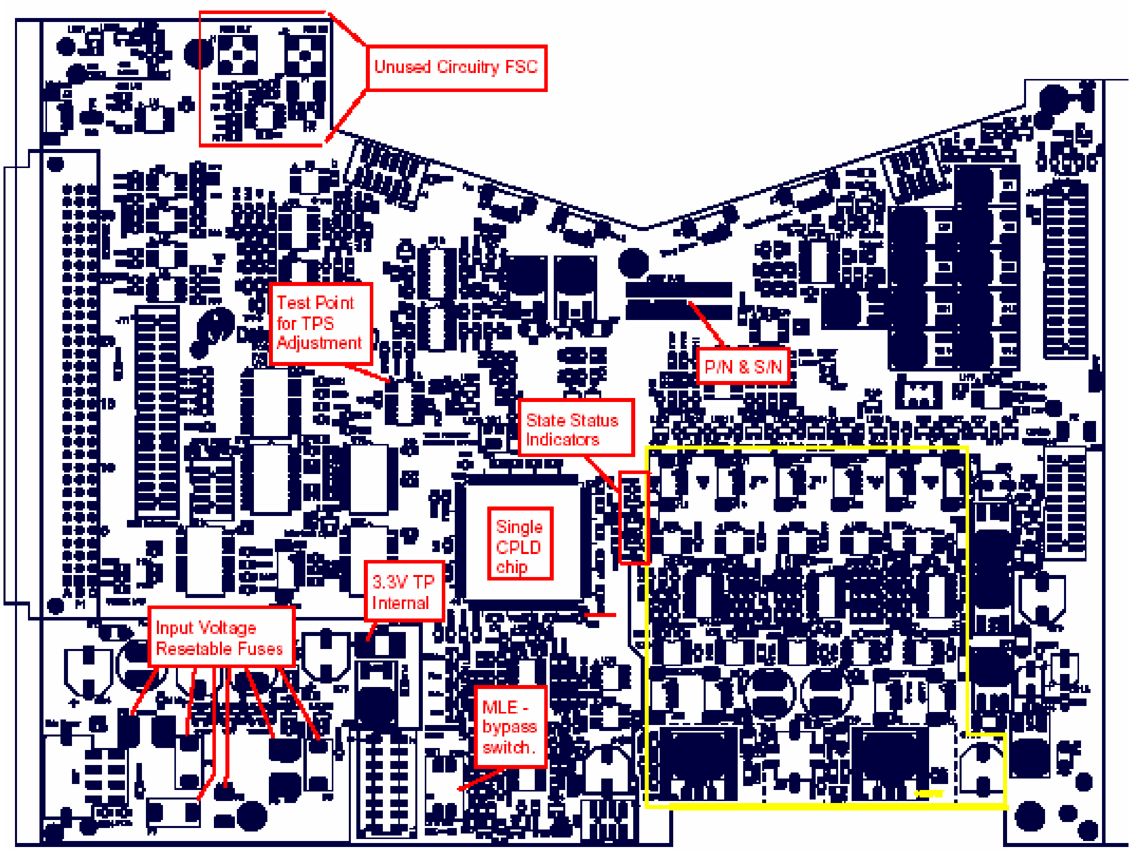
Fluidics Interconnect:
The control board connects to an auxiliary board- the Fluidics Control Board. This board interacts will all of the valves and sensors in the fluidics module. This includes the tube present sensor, the sample arm lever, the sheath pressure gauge, the vacuum pressure gauge, Watson-smith sample pressure regulator, sample air valve, sheath valve, waste valve, debubble valve, lever waste valve, and the tube in tube valve.

The SW1 location on the control board is an override for the CyAn with UV option.

The CPLD requires a minimum of 90% power supply voltage in order to “listen” to the signals sent by Summit. If the power supply voltage dips below this the board will not act.

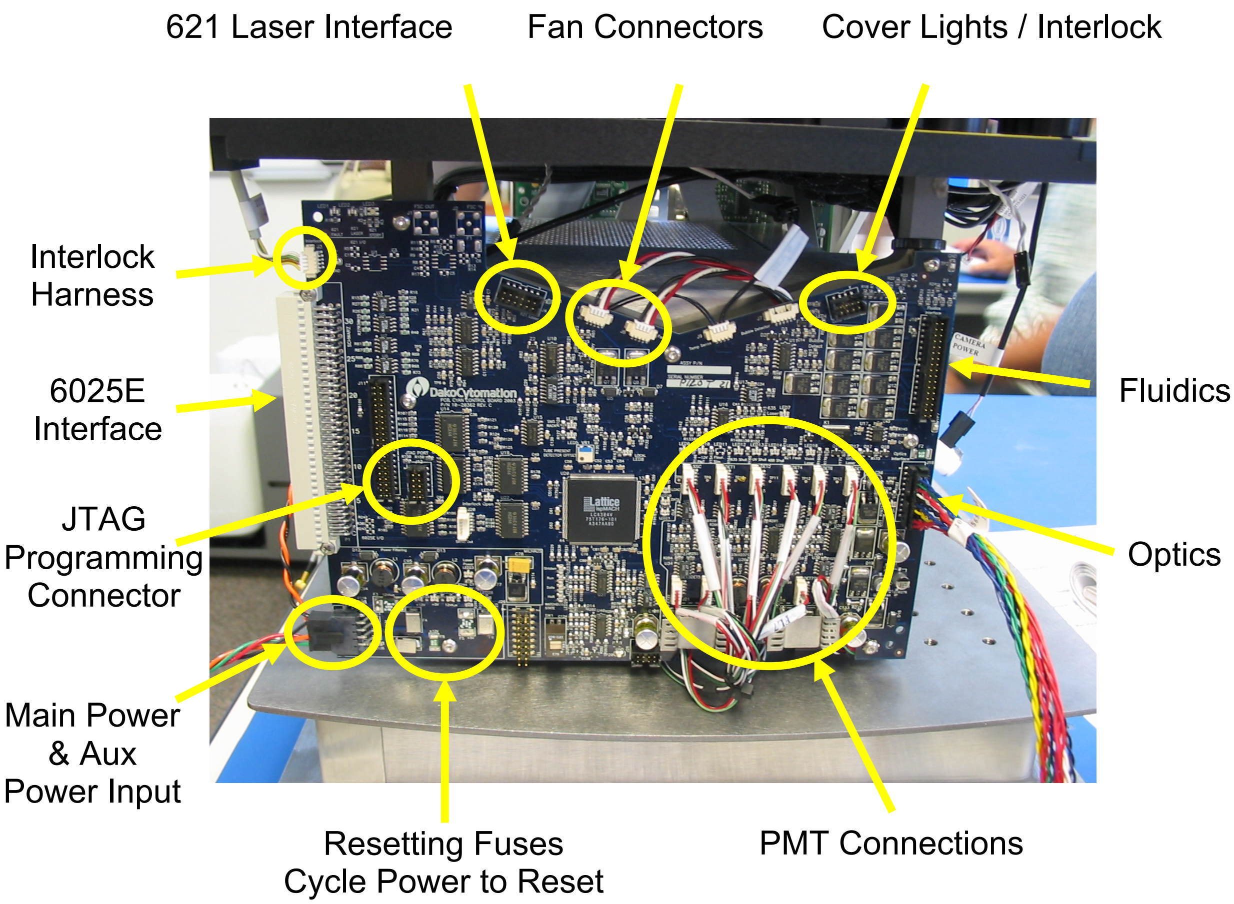


Control board layout with connections.



CyAn ADP Control Board

NOTE: The “MLE bypass switch” should be in the up position for an UV option and in the down position for a non UV options. If this is in the down position for the UV option then the instrument will remain in Halt state (see above section). If it is in the up position for a non UV options then the laser power supply is no longer being monitored



Picture of control board with connections.

Acquisition Electronics (PXI Chassis)

Firmware Theory of Operation

The firmware was designed to be supportable but efficient. The goal was to write as much of the code as possible in C for supportability. All setup and frame-based processing was done in C. Assembly code was used for sample-based processing where efficiency and complete cycle control was needed. Figure 4.1 shows a basic block diagram of the firmware. There are 4 main processes in the DSP. The first process in the chain is the EDMA. This is where the data is read in from the ADCs/FIFOs. The second process is the triggering. This determines the event windows in which measurements will be later made. The third process is the measurements. This is where Linear/Logarithmic height and Linear Area are measured. Master Data is the last process. The Data Master takes all parameters from all the DSPs and organizes them before sending the parameters over the PCI bus.

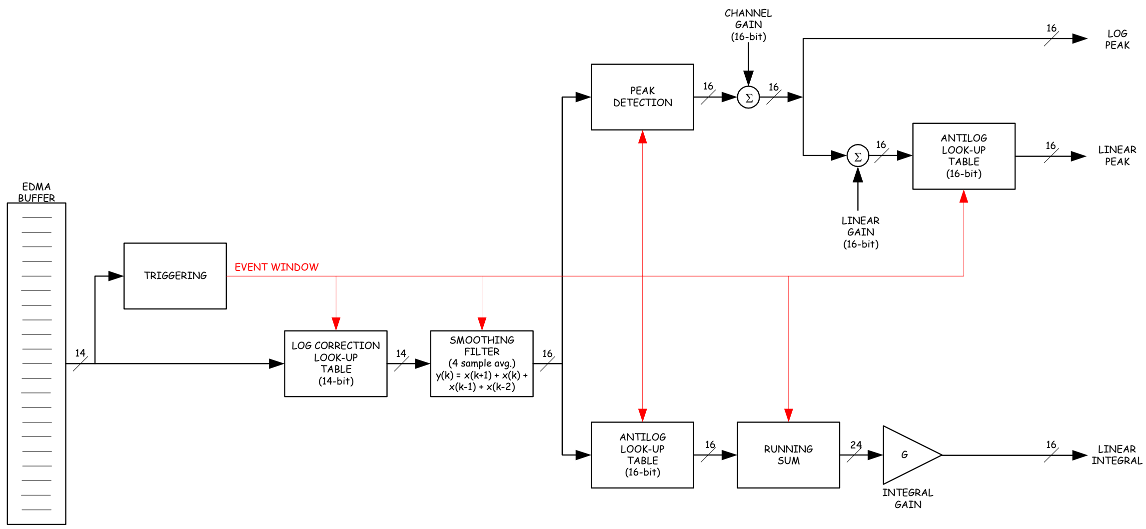


Figure 3.6: Firmware Block Diagram

Triggering

The triggering defines the event window which is later processed for peak and area measurements. The event window is defined by when the signal is equal to or above the threshold. Triggering is done prior to the log correction table and smoothing filter and any measurements to reduce the amount of computation load to the DSP. A pulse width measurement is provided for each event. The pulse width is the number of samples within the event window. Events must not exceed minimum and maximum pulse width requirements to be valid.

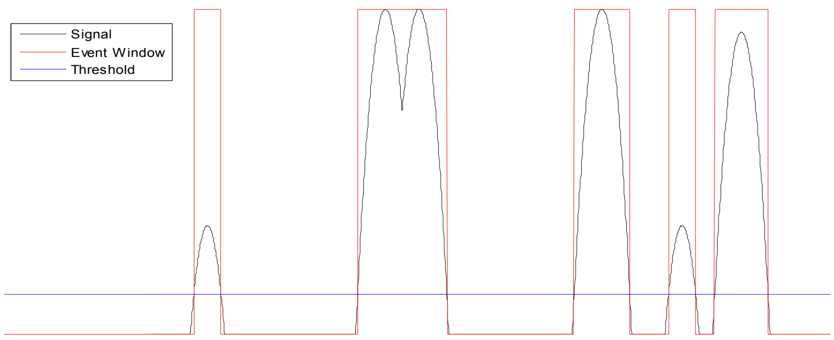
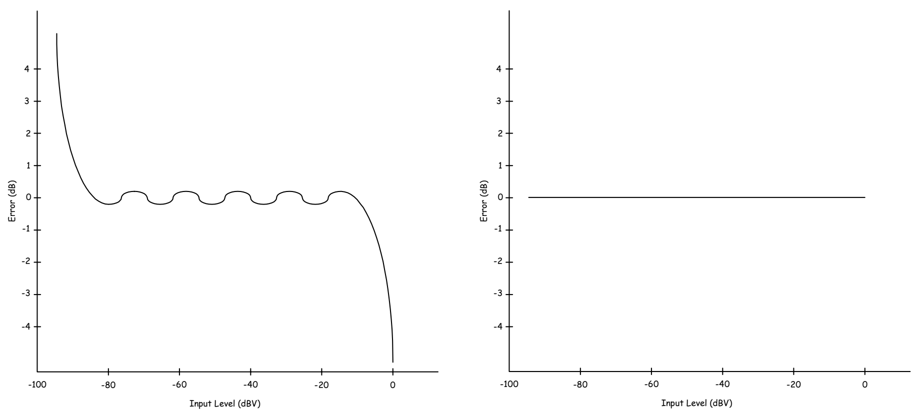


Figure 3.7: Triggering / Event Window Defined

LOG Correction Table

The log correction table is a 14-bit lookup table which corrects for log linearity errors from the log amplifier. The errors are immediately corrected for once the event window has been defined and before all other processing done on the sampled data. The log linearity errors are different for each log amplifier so there is a lookup table for each log amplifier. So each DSP has 4 separate log correction lookup tables.



Log Amp Errors with no Correction Table

Log Amp Errors with Correction Table

Figure 3.8: Log Amplifier Error Correction

Smoothing Filter

A smoothing filter is then used to convert the 14-bit samples into 16-bit samples. This requires a gain of 4 (12dB). The higher frequencies (> 4 MHz) has less than or equal to 0dB.

This filter was used because of low computational cost and for its linear phase properties which causes less distortion to the signal. The formula of the filter is:

$$y(k) = x(k+1) + x(k) + x(k-1) + x(k-2)$$

The above equation can also be re-written as:

$$y(k) = y(k-1) + x(k+1) - x(k-3)$$

This way allows you to take the previous output and add the newest sample and discard the oldest. The cost per filter sample output is 2 adds rather than 3 adds.

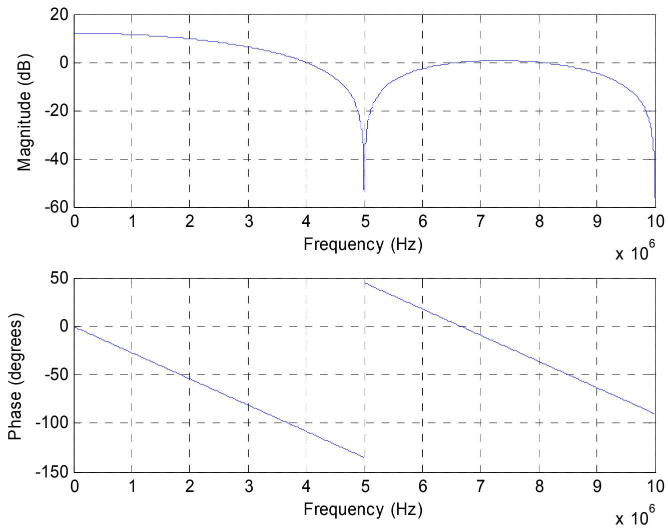


Figure 3.9: Smoothing Filter Frequency Response

Peak and Area Measurements

The log peak is determined by finding the maximum value within the event window. A 16-bit antilog lookup table is used to obtain the linear peak value. A channel gain can be added to the log signal which affects the linear and logged final values. A linear gain can also be added which only affects the linear signal.

The area is calculated by summing all discrete samples within the event window and then multiplying the final 24-bit value with a gain that accounts for the integral scaling, channel gain and the linear gain. All three measurements are supplied for each event.

Frame-Based Processing

The DSP uses the EMIFA bus / EDMA to bring in 1200 samples for each of the 4 channels every 60us. The FIFOs notifies the DSP through the external interrupt 5 (EXT_INT5) that there is a new set of samples to be read. The EDMA reads the samples through the EMIFA bus from the external FIFOs and stores the samples into one of eight EDMA frames. Multiple frames are used to prevent data of being overwritten by the EDMA while the DSP is processing it. This scheme uses 8 frames of data to stagger the EDMA processes and 3 separate DSP software processes: triggering, measurement and data master.

The EDMA is setup as a circular buffer. At initialization the EDMA processes Frame 1. Each time a frame is completed the address of the next frame is loaded. This is repeated until all 8 frames have been filled. The address of the first frame is then loaded allowing the oldest data to be overwritten and the entire process repeats. An interrupt is giving to the DSP at the end of each frame being filled to indicate that a new frame of samples is ready to be processed. Two frames are added to the end of the 8 frames to eliminate pointer checking in the firmware. This was to help reduce the cycle count.

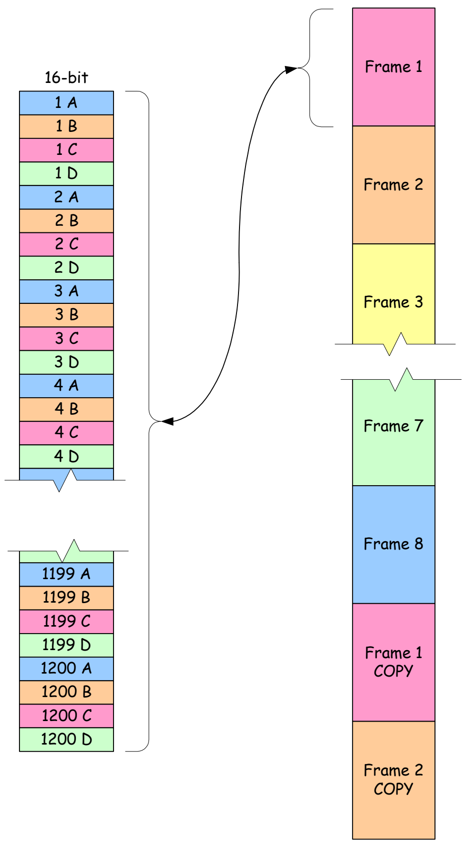


Figure 3.10: Frame-Based Processing

Any time processing is being done on samples such as in the Triggering and Measurement routines, it is necessary to be able to move into the following frame. The reason for this is because of laser delays and events straddling frame boundaries. In this design the frame size must be greater than or equal 2 times the maximum laser delay AND greater than or equal to the maximum pulse width. The processing always begins by taking the address of the frame start and offsetting it by the channel of interest plus the laser delay offset for that channel. Figure 3.11 shows an example of the processing done on frames. In phase one the ADC samples are stored into frame 1 & 9. Triggering processing is done on frames 6-7 and Measurement processing is being done on frames 4-5.

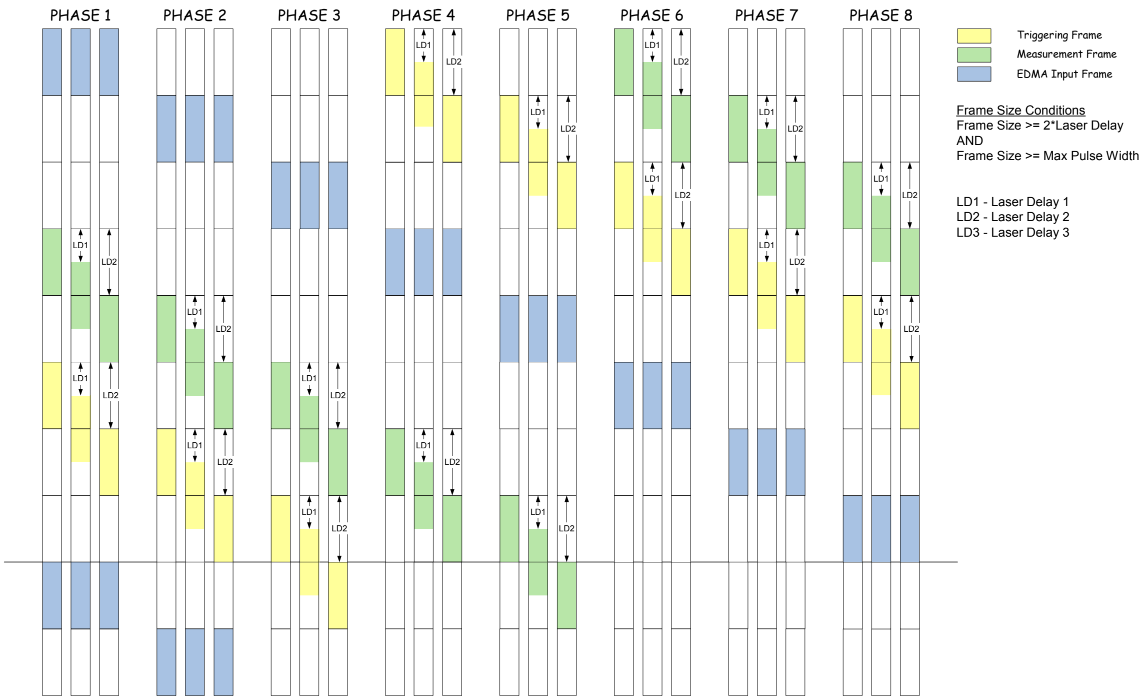


Figure 3.11: EDMA Processes and Measurement Offsets

Triggering Frame

Any one channel can be selected to be the trigger. Within a DSP this can be any of four channels 1-4. Each individual channel can have any one of 3 laser delays. It is possible for an event to straddle a frame border. If the event straddles the frame boundary at the beginning, then the event is considered a part of the previous frame. If the event straddles the boundary at the end of the frame, then the event is part of the present frame. Each frame must meet a min and max pulse width requirement to help eliminate noise and regulate the amount of processing done in the measurement process.

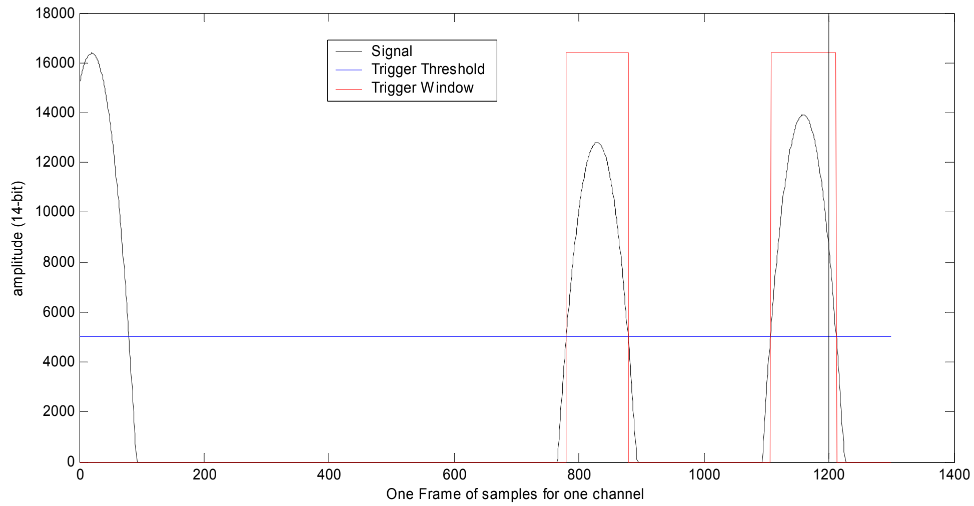


Figure 3.12: Triggering Window Defined

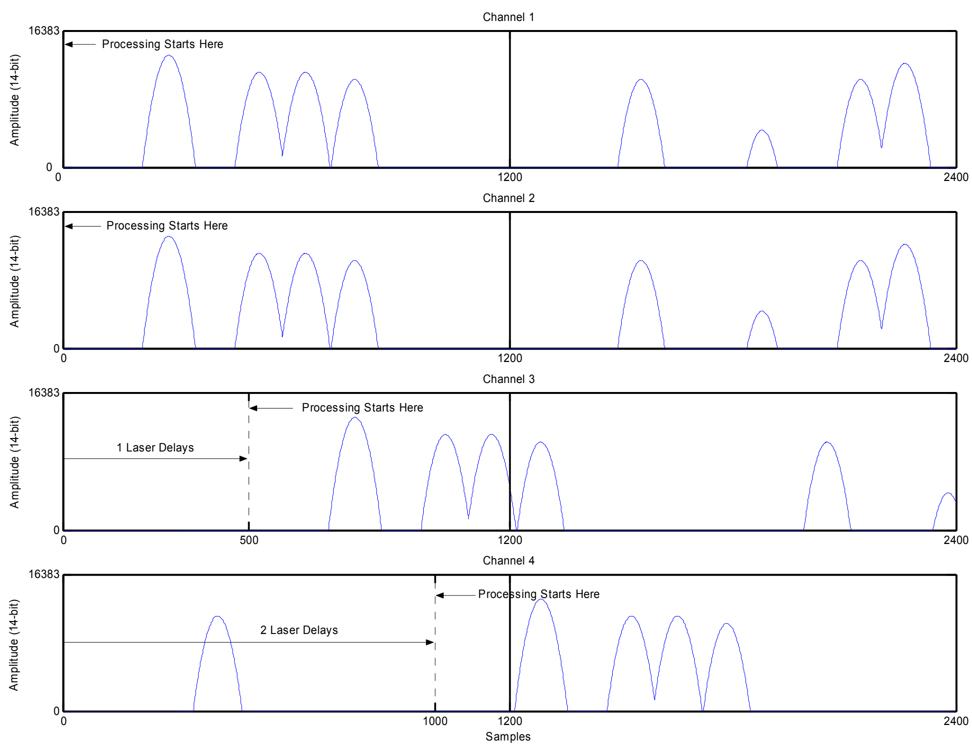


Figure 3.13: Triggering Window Defined

Dynamic Processing Adjustment

This function dynamically schedules event decimation to allow for as many pulses per frame as possible to be processed. The processing is scheduled such that large events will have more decimation during measuring, and smaller events will have less. This is to keep total processing down, while keeping the quantization noise as low as possible. There are 4 levels of decimation based as follows:

Pulse Width (samples):	Max Decimation (samples):
0-89	1
90-179	2
180-269	3
270+	4

If decimation alone cannot get the total processing to be under **nMaxProcAllowed** limit, the events are decremented until it is under. Be aware that if pulses above 360 are allowed, the decimation will be calculated as **pw/90 + 1**, which will allow for rates of 5+. This is done to simplify the code.

Measurement Frame

Measurements are taken for all 4 channels within the DSP once the events have been defined by the triggering for a particular frame. The measurement begins by taking the current measurement frame address + channel number (0 – 3) + laser delay sample offset defined in Figure 4.30 as in the triggering frame. Once measurements for all events have been taken the data is then sent to the Data Master which could be located on different DSP. If the Data Master is located on the same DSP then the data is written in to the Data Master Buffer otherwise it is sent through the PCI bus.

Data Master Frame

Each DSP sends out a frame of measurements to one DSP that is designated at the Data Master. The Data Master first checks to see if each DSP has sent their measurement frame. Then it takes all the measurements for each frame and organizes the data in event groups and sends the event packets one by one to the host PC.

Flow Chart

Each time the DSP is powered up, it is put into reset. The reset initializes the bootloader which copies the main code from the flash memory to internal memory. Once the code has been copied it branches to the main C code. The first part of the main code is to initialization. This sets up all peripherals, interrupts, registers, GPIO, hardware, and the code variables. The main loop has the 3 of the 4 processes: Triggering, Measurement, Data Master. The EDMA will interrupt the processes through an interrupt service routine (ISR) to notify the code that a new frame of data is ready to be processed.

When the ISR is completed it returns back to the main loop and picks up where it left off. When the trigger routine sees that a new frame of buffer is ready it will process it for new events and then notify the software that a new frame of events are ready to be measured. The measurement routine will respond during its next opportunity and then notify the Data Master a new frame of events has been measured. The Data Master waits until it receives all Measurement Frames from each DSP and then sends the event data to the host.

Second-Level Bootloader

The hardware is setup for PCI boot. After reset the DSP is initialized by the PCI host by writing 1k bytes to address 0h through PCI. The DSP is then brought out of reset and then begins execution from address 0h. The firmware exceeds the 1k bytes so a second-level bootloader code is loaded instead. The main firmware is stored in non-volatile flash memory.

The purpose of the second-level bootloader is to copy the firmware stored in flash into internal memory. Once completed the second-level bootloader branches to the main code.

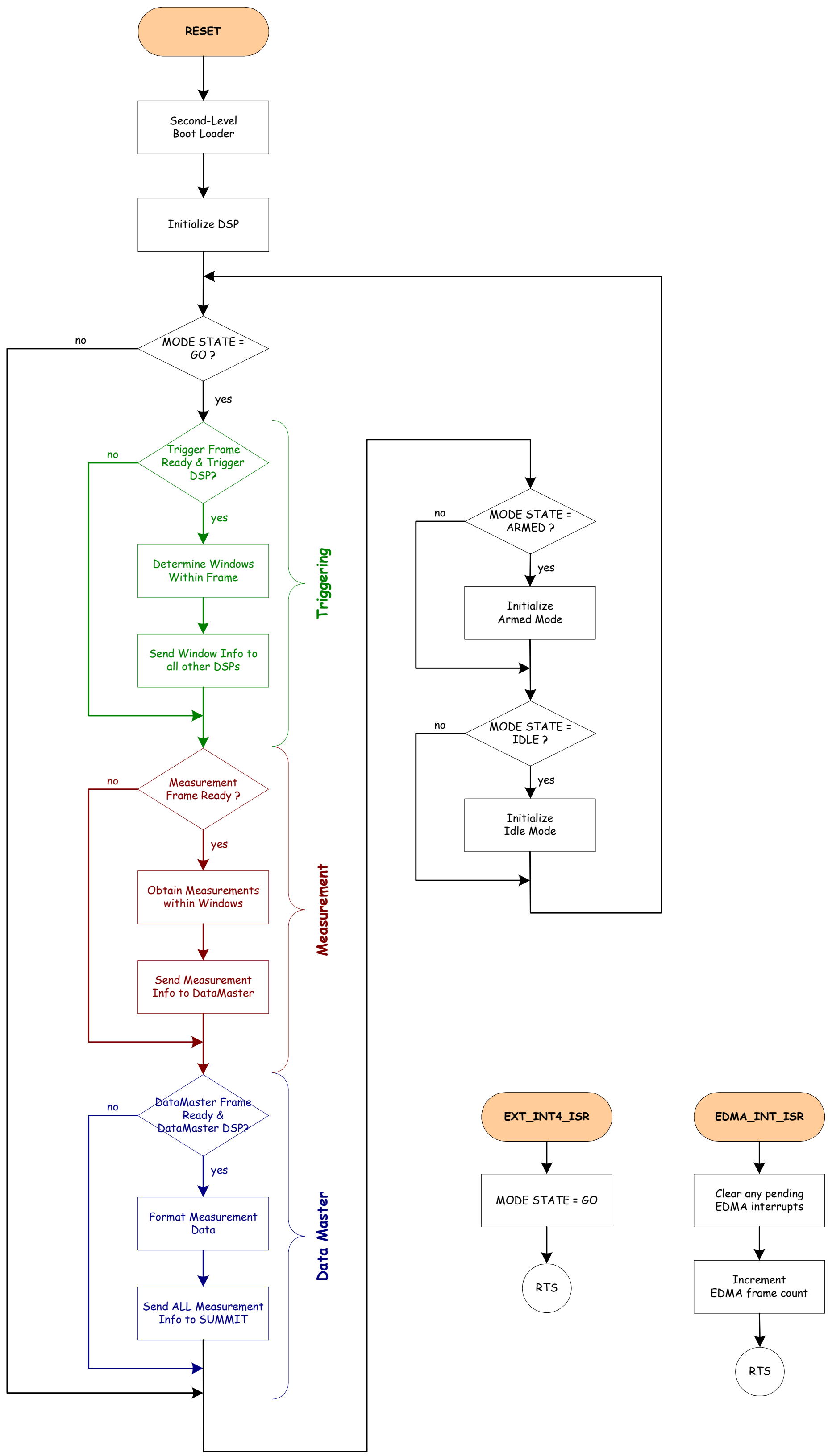
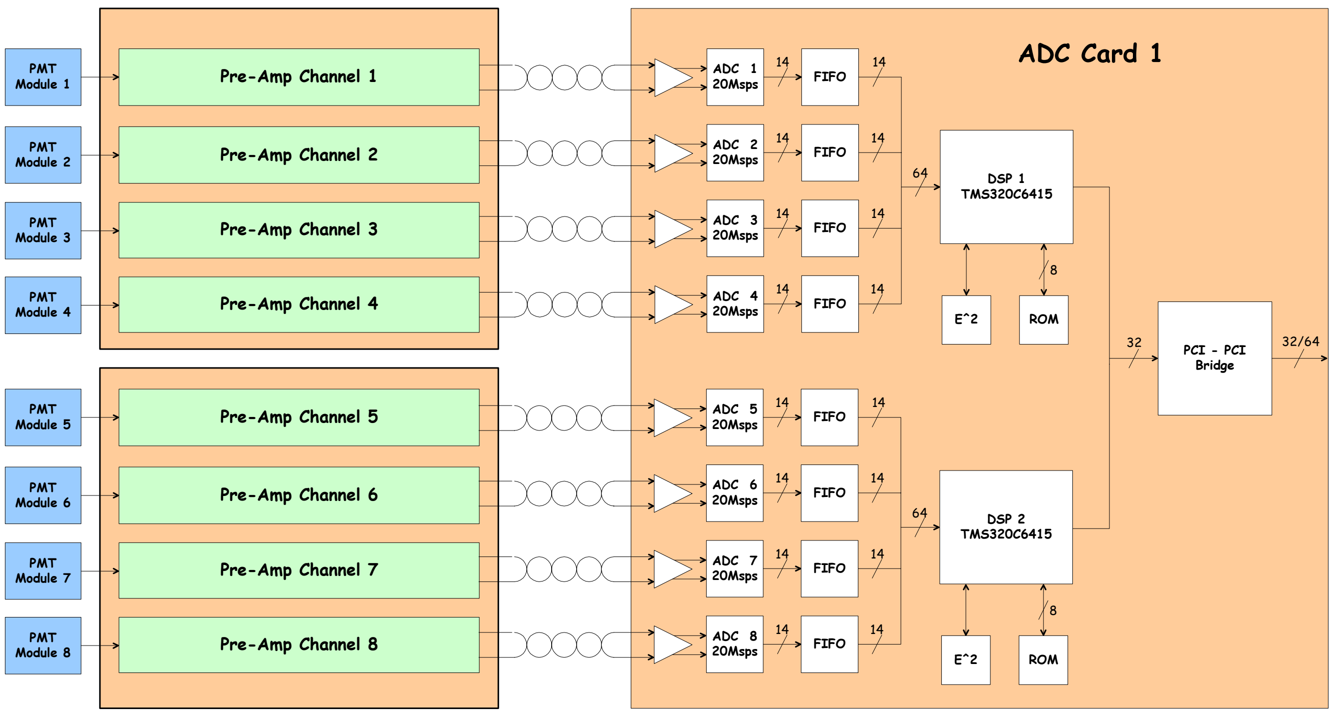
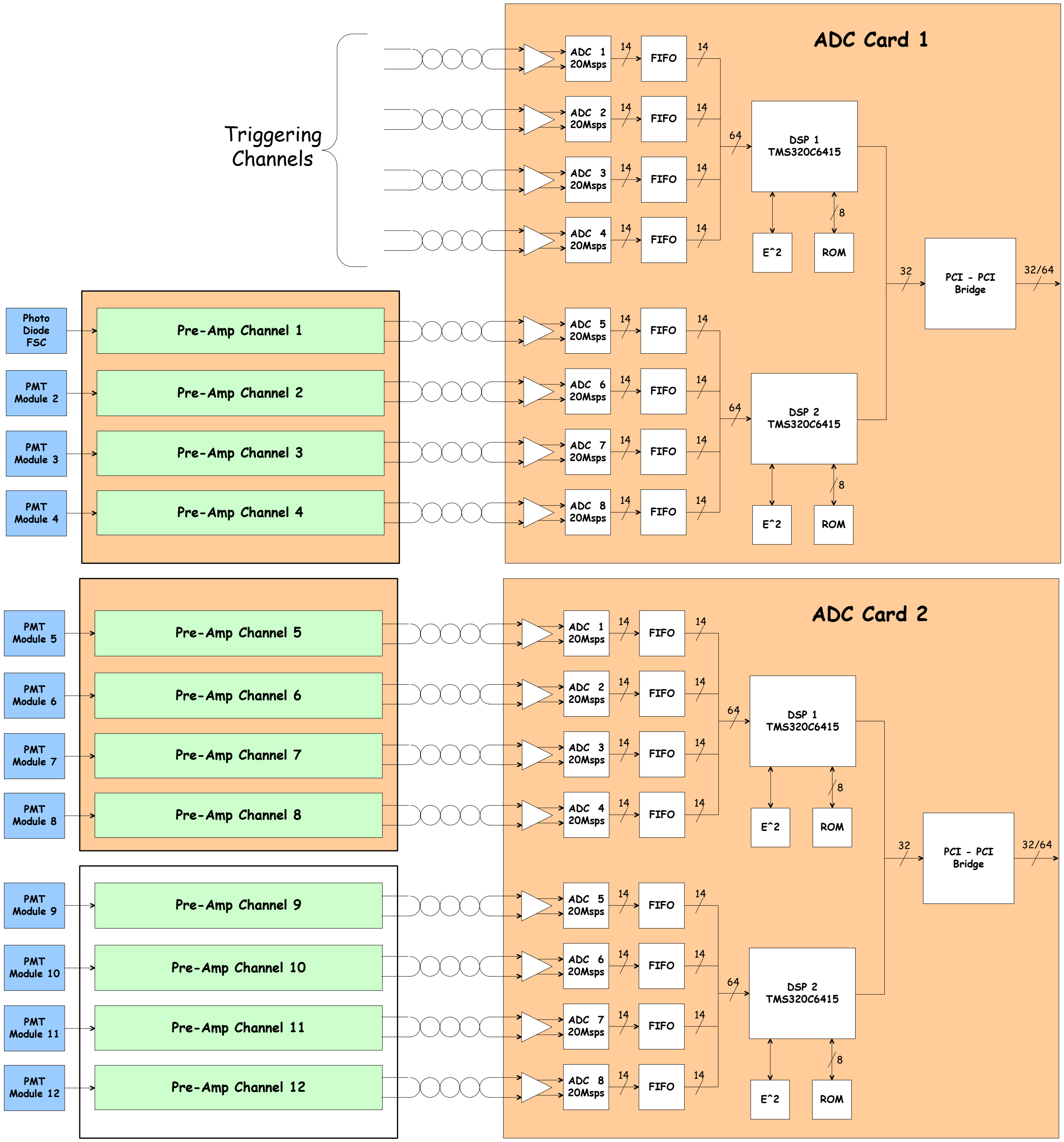


Figure 3.14: Firmware Basic Flow Chart


ADC Card 1
PMT Module 1
PMT Module 2
PMT Module 3
PMT Module 4
PMT Module 5
PMT Module 6
PMT Module 7
PMT Module 8
Pre-Amp Channel 1
Pre-Amp Channel 2
Pre-Amp Channel 3
Pre-Amp Channel 4
Pre-Amp Channel 5
Pre-Amp Channel 6
Pre-Amp Channel 7
Pre-Amp Channel 8
ADC 1 20Msps
ADC 2 20Msps
ADC 3 20Msps
ADC 4 20Msps
ADC 5 20Msps
ADC 6 20Msps
ADC 7 20Msps
ADC 8 20Msps
FIFO
14
64
DSP 1 TMS320C6415
DSP 2 TMS320C6415
E^2
ROM
8
32
PCI - PCI Bridge
32/64



ADC Card 1
Triggering Channels
ADC 1 20Msps
ADC 2 20Msps
ADC 3 20Msps
ADC 4 20Msps
ADC 5 20Msps
ADC 6 20Msps
ADC 7 20Msps
ADC 8 20Msps
FIFO
14
64
32
32/64
8
DSP 1 TMS320C6415
DSP 2 TMS320C6415
E^2
ROM
PCI - PCI Bridge
Photo Diode FSC
PMT Module 2
PMT Module 3
PMT Module 4
Pre-Amp Channel 1
Pre-Amp Channel 2
Pre-Amp Channel 3
Pre-Amp Channel 4
ADC Card 2
PMT Module 5
PMT Module 6
PMT Module 7
PMT Module 8
PMT Module 9
PMT Module 10
PMT Module 11
PMT Module 12
Pre-Amp Channel 5
Pre-Amp Channel 6
Pre-Amp Channel 7
Pre-Amp Channel 8
Pre-Amp Channel 9
Pre-Amp Channel 10
Pre-Amp Channel 11
Pre-Amp Channel 12

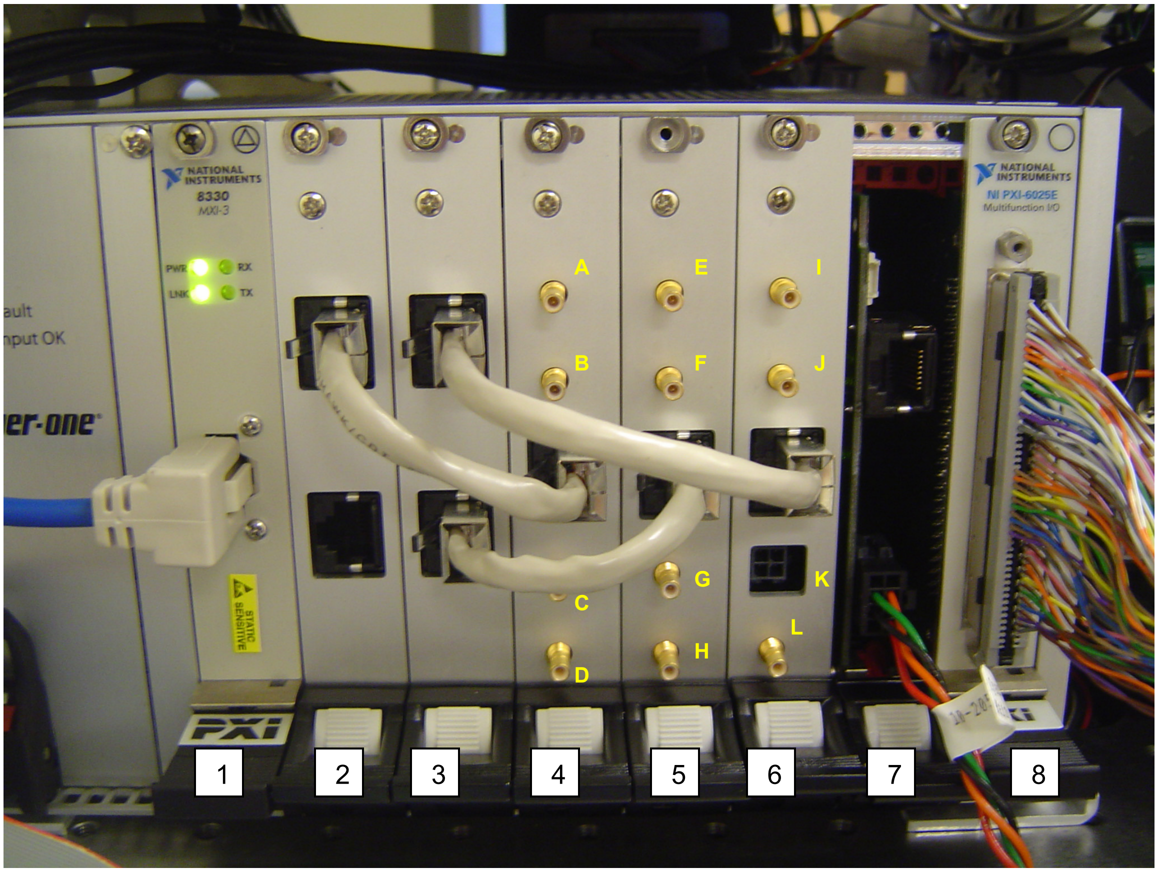


Figure 3.15: PXI Electronics without SMB Connections

Figure 3.15 shows the PXI electronics without the SMB connections in place. The electronics consist of:

1. **PXI MXI card.** This card is the bridge between the acquisition electronics and the computer. The blue communication cable makes the connection from this card to the PCI MXI card in the computer.

2. **Digital Signal Processor (DSP)/Analog to Digital Converter (ADC) Board 1.** This has two DSPs per board with 4 channels per DSP. There fore 8 signals can be processed by this board. The top channel is connected to the first Preamplification board (4). The bottom channel is left empty, but will be used for multi channel triggering in the future.

3. **Digital Signal Processor (DSP)/Analog to Digital Converter (ADC) Board 2.** This has two DSPs per board with 4 channels per DSP. There fore 8 signals can be processed by this board. The bottom channel is connected to the second Preamplification board (5) and the top channel is connected to the third Preamplificaton board (6).

4. **Preamplification (Preamp) Board 1.** This board can process 4 signals: FL7, 8, 9, and10 (note: FL10 not used).

5. **Preamplification (Preamp) Board 2.** This board can process 4 signals: FL3, 4, 5, and 6.

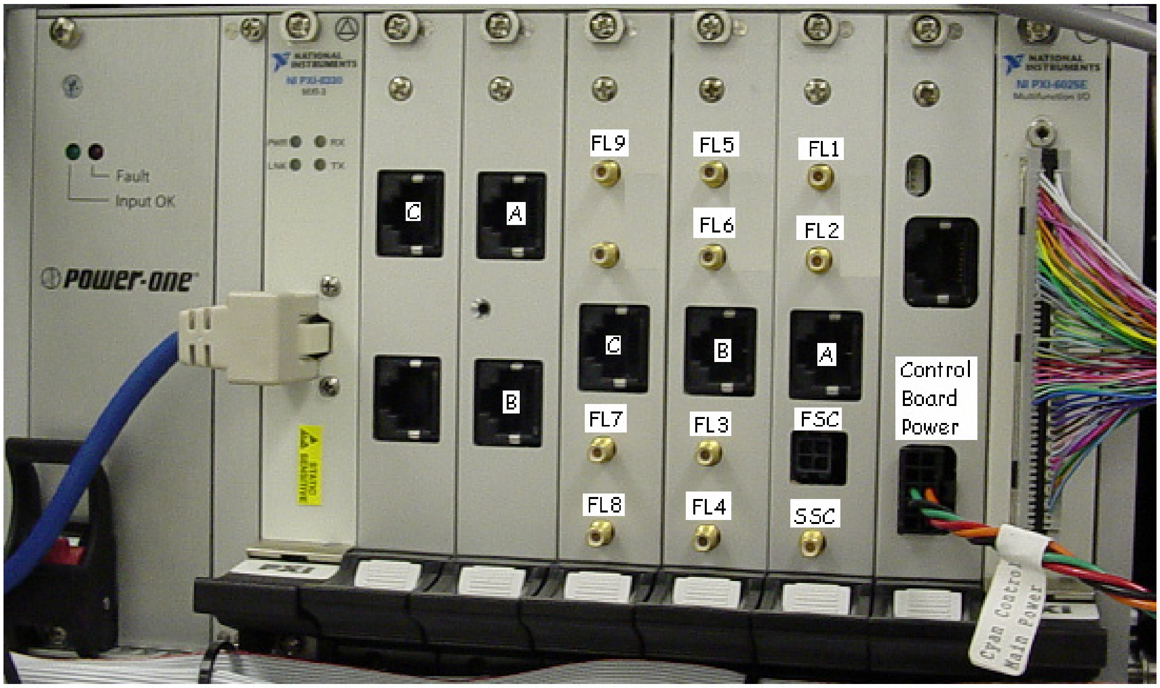
6. **Preamplification (Preamp) Board 3.** This board can process 4 signals: FSC, SSC, FL1 and FL2. Note how the FSC channel has a different connection. This is due to the FSC signal coming from a photodiode instead of a PMT and the signal requiring additional processing. Due to this difference, only the FSC signal can go into this channel and the FSC signal cannot be run through any of the other channels.

7. **Auxiliary (Aux) Board.** This board contains the Auxiliary power and the electronics for multi channel triggering. Note: Currently the multi channel triggering is not in operation.

8. **6025E Card.** This card carries all the signals to and from the CyAn control board for the operation of the instrument.

Figure 3.15 also shows the normal input locations for the BNC connections into the Preamp boards.

A. FL9	D. FL8	G. FL3	J. FL2
B. FL10 (currently not used)	E. FL5	H. FL4	K. FSC
C. FL7	F. FL6	I. FL1	L. SSC



A = 10-20690 3.25" RJ45 Interconnect
B = 10-20689 2.50" RJ45 Interconnect
C = 10-20689 2.50" RJ45 Interconnect

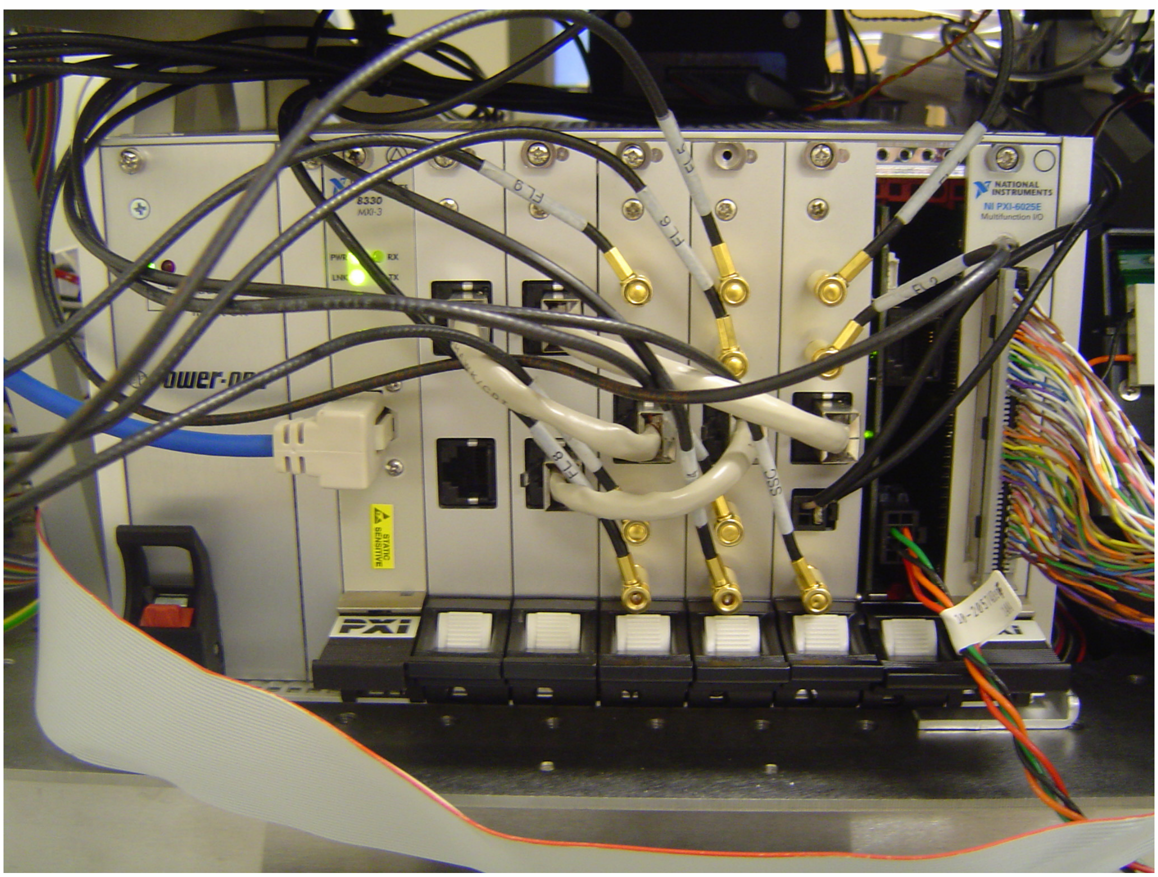
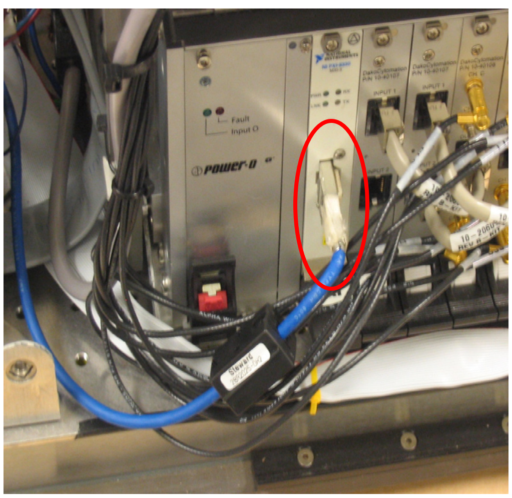


Figure 3.16: PXI Electronics with SMB Connections

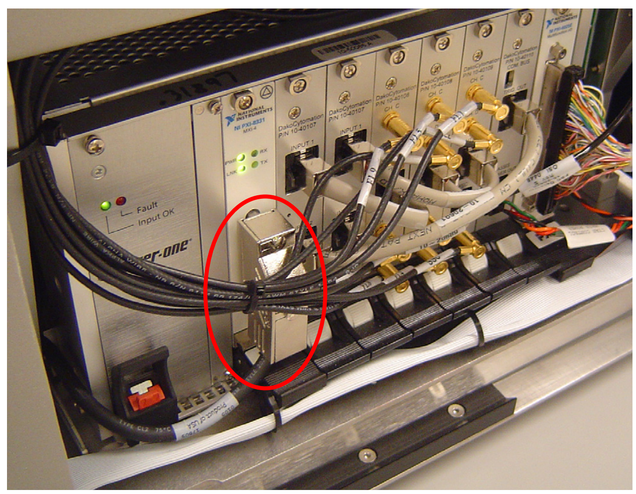
The CyAn ADP with O2 Optics has the MXI-4 boards while the CyAn ADP with out the O2 Optics has the MIX-3 bards. Physically these boards appear the same. There is one board in the CyAn (the PXI MXI-3 or PXI MXI-4) and one board in the computer (the PCI MXI-3 or PCI MXI-4). Only a PCI MXI-3 board will work with a PXI MXI-3 board and the same is true for the MXI-4 boards. Therefore if a MXI-3 board is being replaced it needs to be replaced with the same model of board.

One physical difference between a set of MXI-3 boards and MXI-4 boards is the connecting communications cable. For the MXI-3 boards it will be a 2 meter cable that is blue in color. For the MXI-4 boards it will be a 3 meter cable that is black in color.

The reason for the change to MXI-4 boards is these boards should be even more stable then the MXI-3 boards and the connector on the communications cable is much sturdier.



MXI-3 Connection at PXI Chassis



MXI-4 Connection at PXI Chassis

Lasers

For complete information on the lasers, please refer to their individual manuals. These can be obtained in electronic format from the Global Instrument Support Group.

Coherent Enterprise II 621 Laser – 150 mW at 488 nm and up to 60 mW at 351 (UV) nm
Dako Red Diode laser – 27 mW at 635 nm
Coherent 488 OPSL Diode Laser – 20 mW at 488 nm
Coherent 405 nm Compass Laser- 25 mW at 405 nm

The control board for the Coherent 488 laser must have the dip switch located at position SW3 (A in figure 3.17) set to four (4) in order to autostart. If a new laser does not work immediately, it may be a result of this dip switch being in the wrong position.

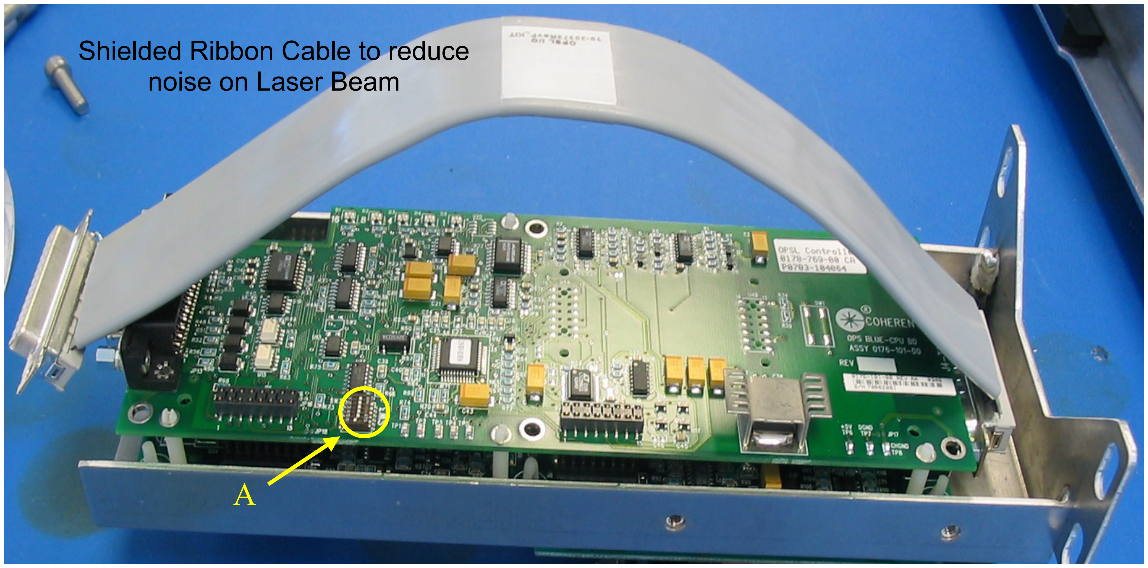
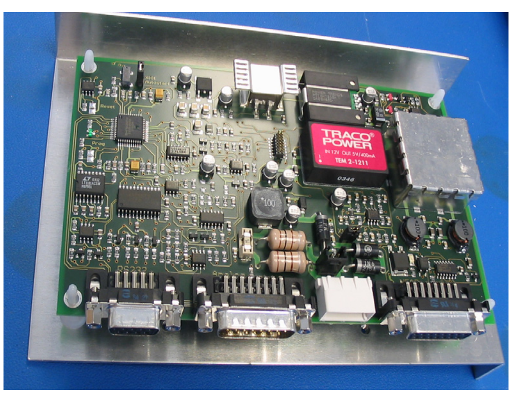


Figure 3.17: Control Board for the Coherent 488 laser.

Coherent Violet Diode Laser – 25 mW at 405 nm

For the Coherent Violet Diode Laser, the control board must have its jumper set on both pins in order to autostart. If a new laser does not work immediately, check that the jumper is set over both pins on the control board (figure 3.18).



Diode Laser Control Board

Jumper on Control Board

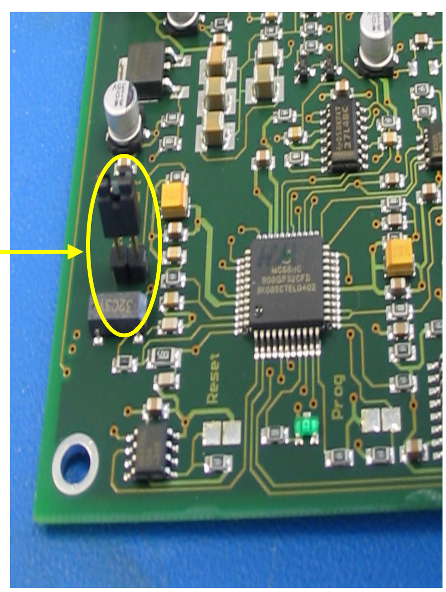


Figure 3.18: Coherent Violet Diode Laser Control Board

Laser Installation

Each Laser requires a **dedicated** circuit for maximum instrument stability.

Notes for water-cooled lasers and in-house water lines:

City water directly to lasers is not recommended due to poor water quality, and possible temperature and pressure fluctuations. Heat exchangers are suggested for use with all water-cooled Lasers.

Customer is responsible for providing all plumbing, pressure gauges, shut-off valves, and fittings for in-house water lines, as well as any “remote” plumbing/connections for heat exchangers.

Lasers connect to in-house water lines and/or heat exchangers via ¾” garden hose type fittings.

New lasers purchased from Dako include above hoses, and a 60 micron particulate filter.

WATER QUALITY IS OF EXTREME IMPORTANCE! Use of distilled water with resistivity of 5kOhm-2MOhm is required for optimal laser tube performance. Deionized water is not recommended for laser cooling due to its potential for laser tube corrosion. Poor water quality can void the laser’s warranty.

Water temperature must be above the dew point of the ambient air to avoid danger of condensation on high voltage laser circuitry.
Pressure differential between inlet and return water lines must be a minimum of 20 psi (138 kPa).

For more comprehensive water guidelines, please visit the Coherent website at http://www.cohr.com/clg/support/clg_ts_water/clg_ts_waterguide1.html

Heat Exchangers

Heat exchangers are needed when operating a CyAn ADP UV option unless an in-house water system is used. Please see “CyAn™ Facility Specs” for specific requirements.

Heat exchangers can be placed within CyAn lab. However, for maximum instrument and environment stability, heat exchangers should be located remotely due to noise and heat dissipation. Remote location cannot exceed 100’ linear, or 20’elevation change.

The Coherent LP-5 heat exchanger is a water-to-air unit which DOES NOT require connection to house water supply. This unit is designed specifically for use with Coherent Enterprise Series Lasers
Coherent LP-5 produces heat (17,000 BTU) and noise and works well placed remotely.
Coherent LP-5 Heat Exchanger uses “standard” 110V outlet.
Coherent LP-5 should have 3 ft of clearance around all sides for effective air flow.
When placed remotely the Coherent LP-5 still needs to be in a temperature controlled environment. Fluctuations in ambient temperature will affect the temperature of water which will affect laser performance.
Coherent LP-20/40/60 Heat Exchangers are generally “hard-wired” to a dedicated 208-240V, 1PH, 10A power source. The customer’s facilities person should be present during the heat exchanger install. Customer can supply other plug/socket combinations if desired.
Coherent LP-20/40/60 heat exchangers are water-to-water units and DO require connection to a house water supply. These units are designed for cooling one, two, and three Lasers respectively.
Please refer to heat exchanger user’s manual for maintenance requirements.

For more specific information on the 488 OPSL, 405 Violet Diode, Enterprise II laser, and the LP5 Heat Exchanger, please refer to their individual manuals. These can be obtained in electronic format from the Global Instrument Support Group.

Summit Workstation

Each Summit Workstation comes with a mouse, keyboard, two LCD monitors, and the CPU.

Section 4

Fluids Management

Sheath Management System Indicators

The Sheath Management System provides 20 - 24 hours of sample run time. As the level of sheath fluid decreases and the level of waste fluid increases, both Summit software and Light Emitting Diodes (LED) indicators on the Sheath Management System front panel will provide fluid level status information and alerts to the user.

SMS indicators can be viewed in the **Sheath Management System** section of the **Instrument** tab in the Control Panel. There are nine indicator lights in the SMS area. When all nine indicator lights are green, all components of the SMS are functioning properly.

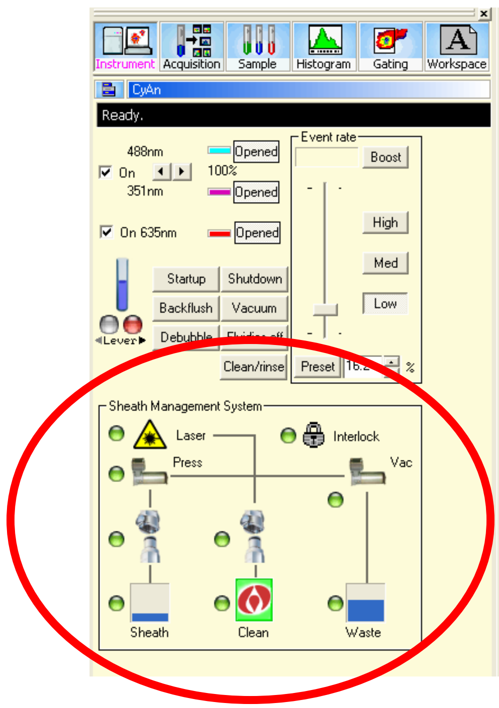


Figure 4.1 – SMS section of Control Panel

If an error condition occurs or status changes for a subsystem, an indicator light will change from green to amber or red. When you place your cursor over the indicator light, a message appears that provides a description of the warning and instructions for how to fix the problem.

The following figures illustrate some of the possible error conditions in the SMS.

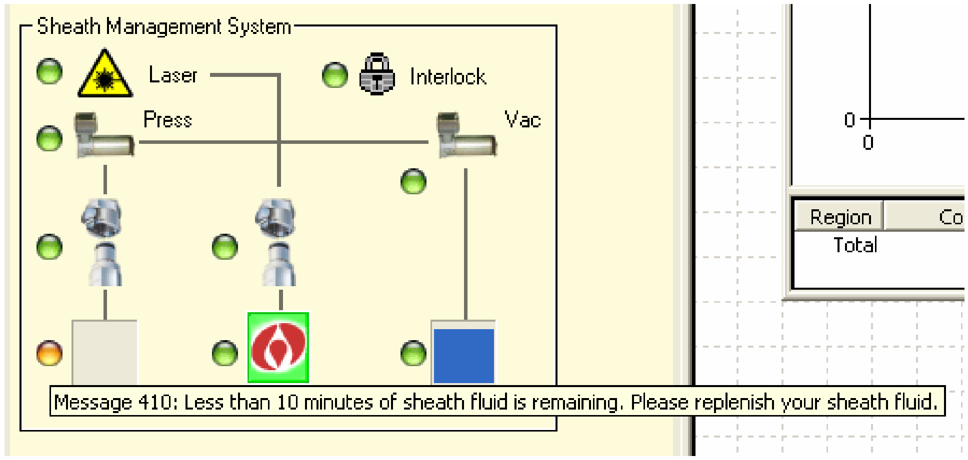


Figure 4.2 – Low sheath fluid warning

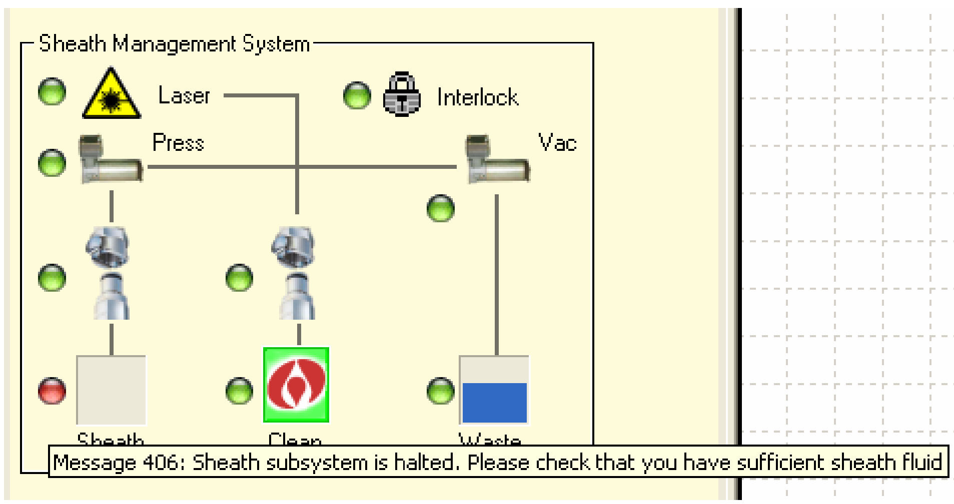


Figure 4.3 – Empty sheath fluid message

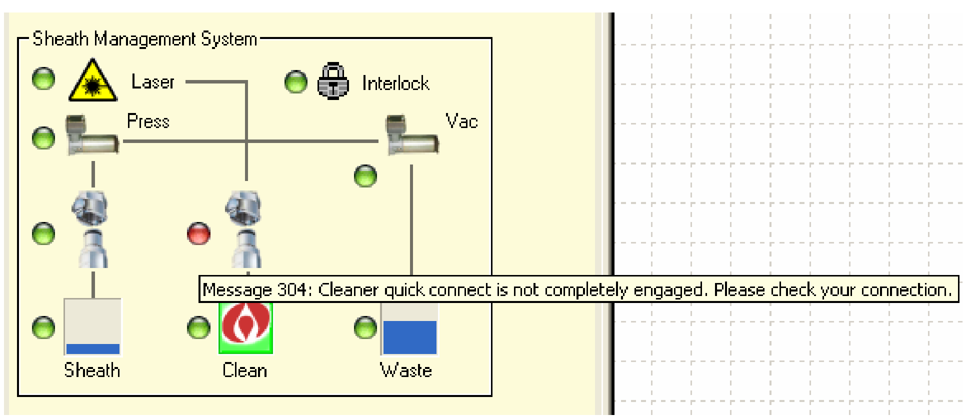
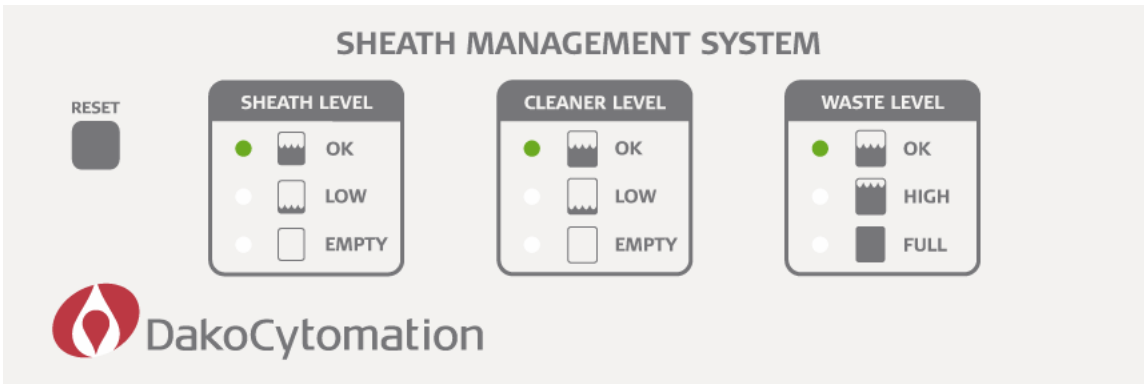


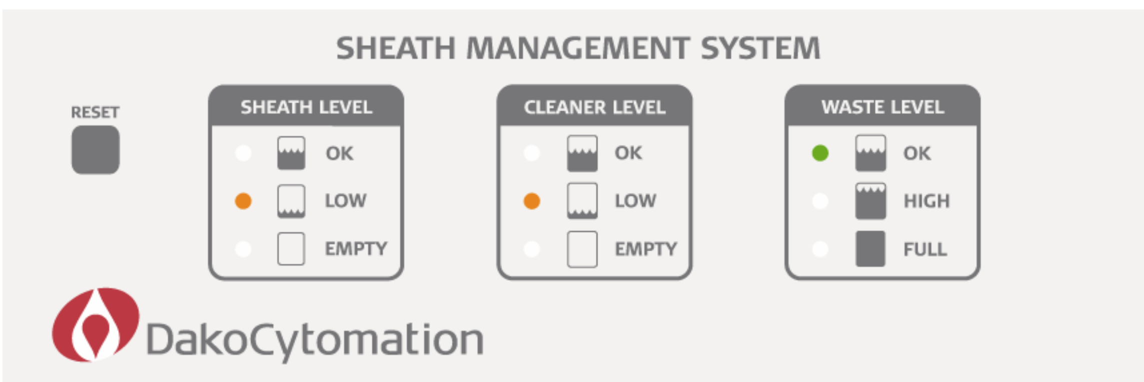
Figure 4.4 – Cleaner quick connect error message

The LED indicators on the front panel of the Sheath Management System also change when fluid levels change in the SMS. Three fluid levels are monitored on the SMS panel: **SHEATH LEVEL**, **CLEANER LEVEL**, and **WASTE LEVEL**.

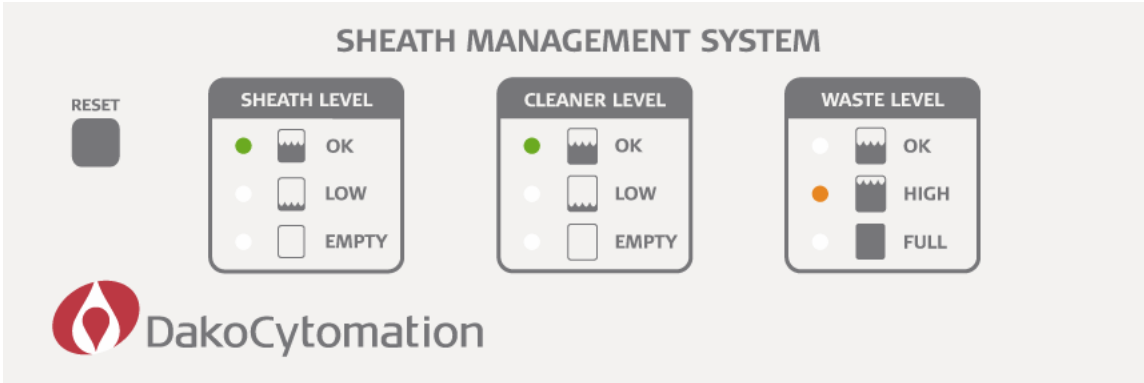
When all fluids are at suitable levels for proper operation of the CyAn ADP, the **SHEATH LEVEL**, **CLEANER LEVEL**, and **WASTE LEVEL** LEDs appear green beside the **OK** label:



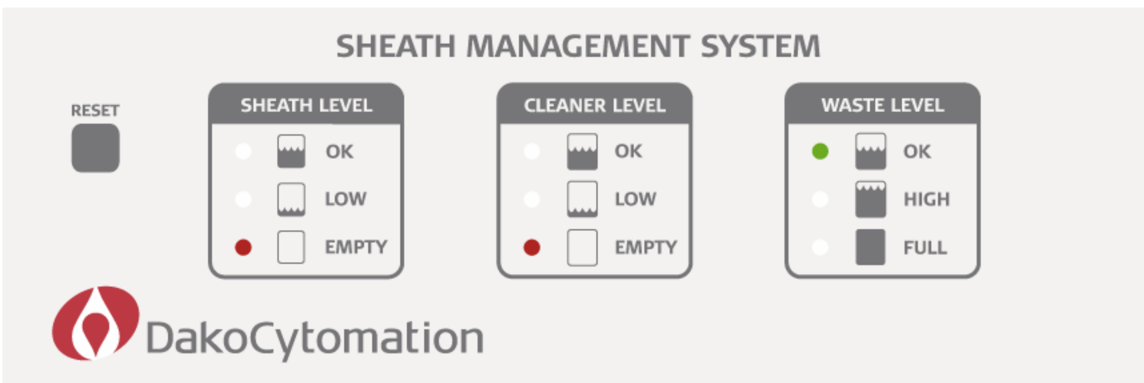
When the sheath level or cleaner fluid level is low, the green **OK** LED changes to a flashing amber or a solid amber LED beside the **LOW** label:



When the waste fluid level is high, the green **OK** LED changes to a flashing amber LED beside the **HIGH** label:



When the sheath or cleaner level changes from low to empty, the flashing amber LED changes to a red LED beside the **EMPTY** label:



When the waste fluid level changes from high to full, the flashing amber LED changes to a red LED beside the **FULL** label:

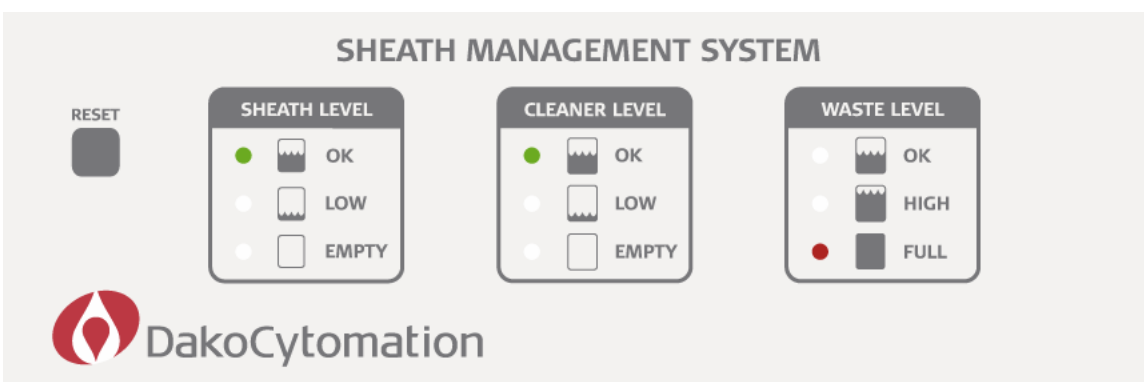
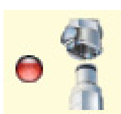
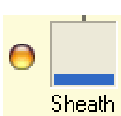
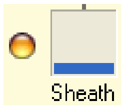
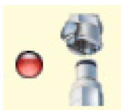
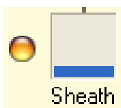
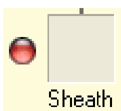
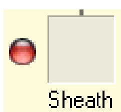
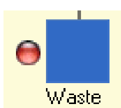
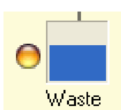
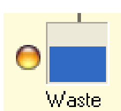
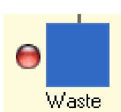
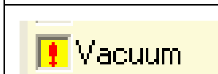
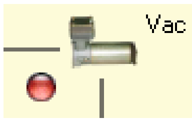


Table 4.1 lists the indicators in the CyAn Control Panel user interface and the message text for each error or status condition.

Table 4.1: CyAn ADP Control Panel Indicators

CyAn Control Panel	Message Text
	Message 101: The 621 Laser has reported a fault. Please check the cooling lines and interlocks on the 621 Laser.
	Message 102: Your CyAn cover is open. The laser shutters have been closed and fluidic system is shutdown. Please close your cover and restart.
	Message 301: Cleaner fluid is low. Please replace the cleaner solution and press the Startup button on the CyAn Control Panel.
	Message 302: Cleaner fluid is empty. Please replace the cleaner solution and press the Startup button on the CyAn Control Panel.
	Message 304: Cleaner quick connect is not completely engaged. Please check your connection.
	Message 305: Internal reservoir overfilled. This will not prevent operation of the instrument but may require future service.
	Message 306: Clean subsystem is halted. Please check that you have sufficient cleaner fluid.
	Message 307: Cleaner subsystem switch error. This will not prevent operation of the instrument but may require future service.
	Message 430: Less than 30 min of sheath fluid is remaining.
	Message 410: Less than 10 minutes of sheath fluid is remaining. Please replenish your sheath fluid.

CyAn Control Panel	Message Text
	Message 401: Internal sheath reservoir level is low. CyAn will stop soon. Please replenish your sheath fluid.
	Message 402: Out of sheath fluid. Replenish sheath and press the Startup button on the CyAn Control Panel.
	Message 404: Sheath quick connect is not completely engaged. Please check your connection.
	Message 405: Internal reservoir overfilled. This will not prevent operation of the instrument but may require future service.
	Message 406: Sheath subsystem is halted. Please check that you have sufficient sheath fluid.
	Message 407: Sheath subsystem switch error. Service maybe required.
	Message 705: Waste subsystem is halted. Please check waste tank level.
	Message 730: Less than 30 min until the waste container is full.
	Message 710: Less than 10 min until the waste container is full. Please empty the waste container.
	Message 700: Waste container is full. Please empty the waste container.
	Message 905: Low sheath pressure inside CyAn. Check connection between the CyAn and SMS.
	Message 906: Low vacuum inside CyAn. Check connection between CyAn and SMS.

CyAn Control Panel	Message Text
	Message 997: Low sheath pressure in SMS.
	Message 998: Waste subsystem halted due to loss of vacuum. If during operation, please check vacuum pump, waste quick connect or waste tank level.
<Message in top portion of Control Panel>	Message 999: SMS Power fault. Reset SMS to continue. If this problem reoccurs, please call Dako for technical support.

Log Files

Most of the SMS errors as well as different operations performed by the customer are logged by Summit for reference. The current log file is named Cyan.log, and can be found in the following directory: C:\Program Files\Dako\Summit <version>\Log. After the file size is approximately 500KB, the log file will be renamed to include the date that the file was saved in the file name.

The current log file will not be dated. It can only be opened if Summit is closed (not in operation). It will show the most recent action on the CyAn ADP (figure 4.5).

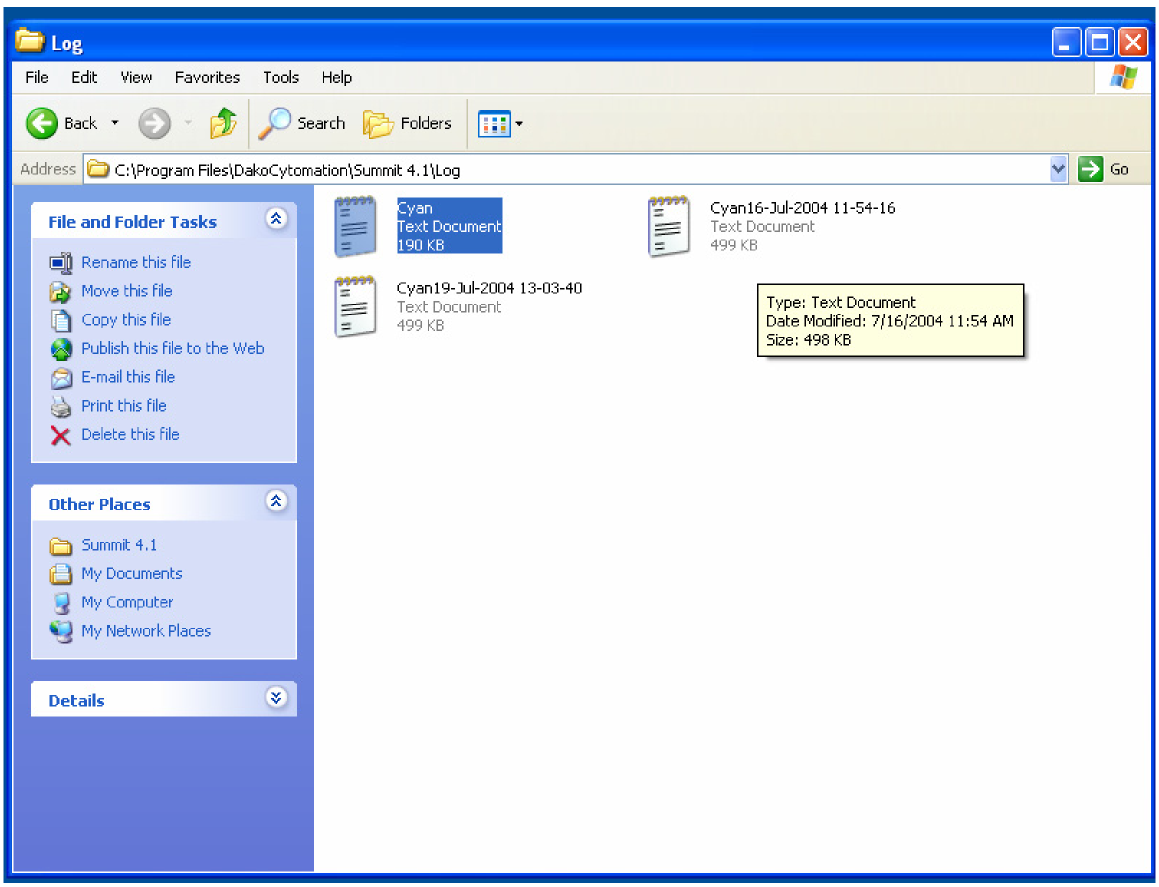


Figure 4.5

When the current log file reaches 499KB, it will be saved with the current date. These files can be opened at any time – even if Summit is open (figure 4.6).

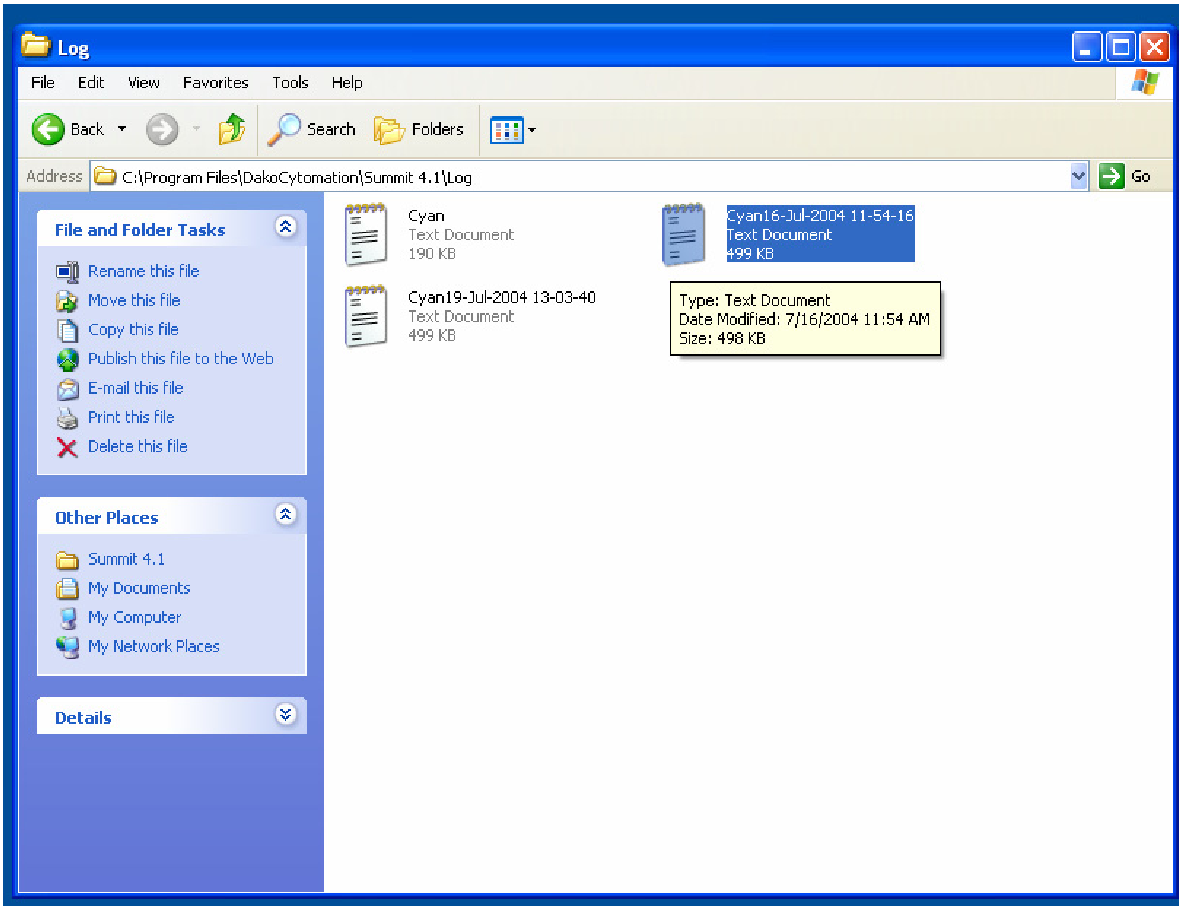
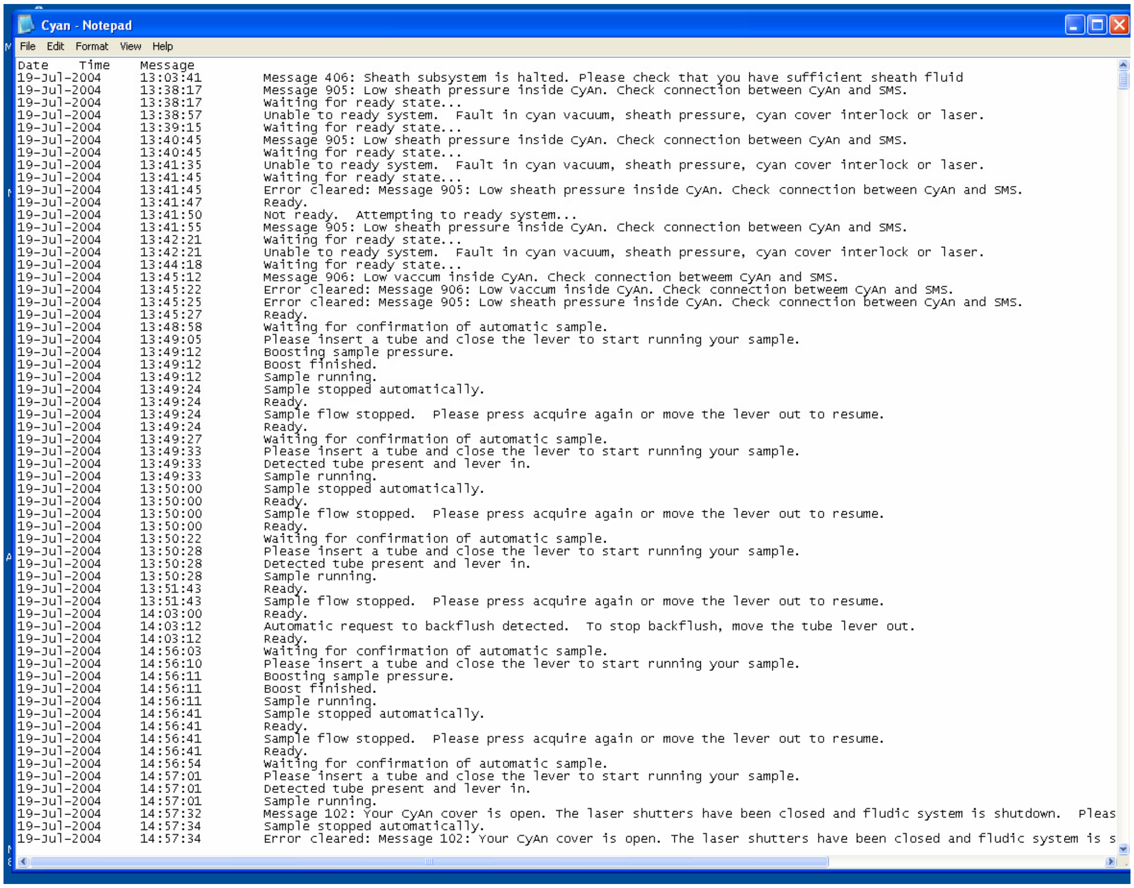


Figure 4.6

Once open, the files show the latest actions or errors performed on the instrument. These can be used to help determine if a fault is occurring intermittently or see how the customer is using the instrument. The files can also be sent by email to individual for help (figure 4.7).




```
Date       Time        Message
19-Jul-2004  13:03:41  Message 406: Sheath subsystem is halted. Please check that you have sufficient sheath fluid
19-Jul-2004  13:38:17  Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004  13:38:17  Waiting for ready state...
19-Jul-2004  13:38:57  Unable to ready system.  Fault in cyan vacuum, sheath pressure, cyan cover interlock or laser.
19-Jul-2004  13:39:15  Waiting for ready state...
19-Jul-2004  13:40:45  Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004  13:40:45  Waiting for ready state...
19-Jul-2004  13:41:35  Unable to ready system.  Fault in cyan vacuum, sheath pressure, cyan cover interlock or laser.
19-Jul-2004  13:41:45  Waiting for ready state...
19-Jul-2004  13:41:45  Error cleared: Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004  13:41:47  Ready.
19-Jul-2004  13:41:50  Not ready.  Attempting to ready system...
19-Jul-2004  13:41:55  Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004  13:42:21  Waiting for ready state...
19-Jul-2004  13:42:21  Unable to ready system.  Fault in cyan vacuum, sheath pressure, cyan cover interlock or laser.
19-Jul-2004  13:44:18  Waiting for ready state...
19-Jul-2004  13:45:12  Message 906: Low vaccum inside CyAn. Check connection betweem CyAn and SMS.
19-Jul-2004  13:45:22  Error cleared: Message 906: Low vaccum inside CyAn. Check connection betweem CyAn and SMS.
19-Jul-2004  13:45:25  Error cleared: Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004  13:45:27  Ready.
19-Jul-2004  13:48:58  Waiting for confirmation of automatic sample.
19-Jul-2004  13:49:05  Please insert a tube and close the lever to start running your sample.
19-Jul-2004  13:49:12  Boosting sample pressure.
19-Jul-2004  13:49:12  Boost finished.
19-Jul-2004  13:49:12  Sample running.
19-Jul-2004  13:49:24  Sample stopped automatically.
19-Jul-2004  13:49:24  Ready.
19-Jul-2004  13:49:24  Sample flow stopped.  Please press acquire again or move the lever out to resume.
19-Jul-2004  13:49:24  Ready.
19-Jul-2004  13:49:27  Waiting for confirmation of automatic sample.
19-Jul-2004  13:49:33  Please insert a tube and close the lever to start running your sample.
19-Jul-2004  13:49:33  Detected tube present and lever in.
19-Jul-2004  13:49:33  Sample running.
19-Jul-2004  13:50:00  Sample stopped automatically.
19-Jul-2004  13:50:00  Ready.
19-Jul-2004  13:50:00  Sample flow stopped.  Please press acquire again or move the lever out to resume.
19-Jul-2004  13:50:00  Ready.
19-Jul-2004  13:50:22  Waiting for confirmation of automatic sample.
19-Jul-2004  13:50:28  Please insert a tube and close the lever to start running your sample.
19-Jul-2004  13:50:28  Detected tube present and lever in.
19-Jul-2004  13:50:28  Sample running.
19-Jul-2004  13:51:43  Ready.
19-Jul-2004  13:51:43  Sample flow stopped.  Please press acquire again or move the lever out to resume.
19-Jul-2004  14:03:00  Ready.
19-Jul-2004  14:03:12  Automatic request to backflush detected.  To stop backflush, move the tube lever out.
19-Jul-2004  14:03:12  Ready.
19-Jul-2004  14:56:03  Waiting for confirmation of automatic sample.
19-Jul-2004  14:56:10  Please insert a tube and close the lever to start running your sample.
19-Jul-2004  14:56:11  Boosting sample pressure.
19-Jul-2004  14:56:11  Boost finished.
19-Jul-2004  14:56:11  Sample running.
19-Jul-2004  14:56:41  Sample stopped automatically.
19-Jul-2004  14:56:41  Ready.
19-Jul-2004  14:56:41  Sample flow stopped.  Please press acquire again or move the lever out to resume.
19-Jul-2004  14:56:41  Ready.
19-Jul-2004  14:56:54  Waiting for confirmation of automatic sample.
19-Jul-2004  14:57:01  Please insert a tube and close the lever to start running your sample.
19-Jul-2004  14:57:01  Detected tube present and lever in.
19-Jul-2004  14:57:01  Sample running.
19-Jul-2004  14:57:32  Message 102: Your CyAn cover is open. The laser shutters have been closed and fludic system is shutdown.  Pleas
19-Jul-2004  14:57:34  Sample stopped automatically.
19-Jul-2004  14:57:34  Error cleared: Message 102: Your CyAn cover is open. The laser shutters have been closed and fludic system is s
```

Figure 4.7

Date	Time	Message
19-Jul-2004	13:03:41	Message 406: Sheath subsystem is halted. Please check that you have sufficient sheath fluid
19-Jul-2004	13:38:17	Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:38:17	Waiting for ready state...
19-Jul-2004	13:38:57	Unable to ready system. Fault in CyAn vacuum, sheath pressure, CyAn cover interlock or laser.
19-Jul-2004	13:39:15	Waiting for ready state...
19-Jul-2004	13:40:45	Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:40:45	Waiting for ready state...
19-Jul-2004	13:41:35	Unable to ready system. Fault in CyAn vacuum, sheath pressure, CyAn cover interlock or laser.
19-Jul-2004	13:41:45	Waiting for ready state...
19-Jul-2004	13:41:45	Error cleared: Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:41:47	Ready.
19-Jul-2004	13:41:50	Not ready. Attempting to ready system...
19-Jul-2004	13:41:55	Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:42:21	Waiting for ready state...
19-Jul-2004	13:42:21	Unable to ready system. Fault in CyAn vacuum, sheath pressure, CyAn cover interlock or laser.
19-Jul-2004	13:44:18	Waiting for ready state...
19-Jul-2004	13:45:12	Message 906: Low vacuum inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:45:22	Error cleared: Message 906: Low vacuum inside CyAn. Check connection between CyAn and SMS.
19-Jul-2004	13:45:25	Error cleared: Message 905: Low sheath pressure inside CyAn. Check connection between CyAn and SMS.

Figure 4.8: Example of a section of a log file.

Changing Out Sheath and Waste Containers

Use the shutdown fluidics procedure to fill and empty sheath and waste containers when running samples.

1. Click **Fluidics Off** on the CyAn Control Panel.

WARNING

Remove the waste container and the sheath container from the cart before emptying or filling. Dispose of the contents of the waste container in accordance with local, state, and federal regulations.

Do not drop the waste or sheath containers on the Sheath Management System. Doing so may result in improper calibration of the load cells.

2. At the waste container, release the quick-connects.

3. Remove the waste container from the cart, remove the lid, and empty. Dispose of the contents of the waste container in accordance with local, state, and federal regulations.

4. Place an appropriate amount and type of disinfectant in the bottom of the waste tank to ensure effective killing action when the tank is full. All biologicals introduced into the waste tank must be in contact with the disinfectant for a minimum of 10 minutes before being discarded.

As an example: If 200mL of regular household bleach (5.25% active chlorine) is placed in the container, this will provide approximately 500ppm available chlorine when the tank's full capacity of 20L has been reached. If the samples you are running will not be effectively killed by this concentration of sodium hypochlorite solution, an increased amount of bleach should be used to achieve effective disinfection concentration. Please keep in mind that diluted sodium hypochlorite solutions begin losing their effectiveness after 24 hours.

5. Replace the lid and tighten, place the container back in its position on the cart, and reconnect the waste container using the quick-connects.

6. Restore the sheath fluid by either replacing the entire sheath cubitainer or refilling the plastic carboy, depending on which type of sheath container is used with your Sheath Management System:

    - If you are using the disposable sheath cubitainer, release the quick-connects. Dispose of the entire cubitainer in accordance with local, state, and federal regulations (for example, recycle the outside cardboard box and place the plastic bladder in an appropriate receptacle). Place a new sheath cubitainer back in its position on the cart. Locate the quick connect inside the box. Spray the quick connect with 70% ethanol. Reconnect the cubitainer to the SMS cart.

- If you are using the plastic carboy, release the quick-connects. Remove the carboy from the cart, loosen the lid, and fill with particulate-free deionized water (dH2O) or a suitable sheath fluid. Seal the lid, and place the container back in its position on the cart. Spray the quick connect with 70% ethanol. Reconnect the sheath container to the SMS cart using the quick-connects.

7. Click **Startup** on the Cyan Control Panel.

Replacing Cleaner Fluid

1. Release the cleaner cubitainer quick-connect.
2. Unscrew the cap from the spent cleaner cubitainer. Retain this cap for use on the new cubitainer.
3. Dispose of the entire cleaner cubitainer in accordance with local, state, and federal regulations.
4. Remove the cardboard punch-out on a new cleaner cubitainer. Holding onto the ring around the cap, pull up on the lid so that the lid extends up to the cardboard.
5. Remove the cap and replace it with the cap from the previous cubitainer.
6. Place a new cleaner cubitainer back in its position on the cart. Spray the quick-connect with 70% ethanol. Reconnect the cubitainer to the SMS cart.

Section 5

Alignment

CyAn Alignment

Alignment will be split up into a coarse and fine alignment. Coarse Alignment includes translation and position of the lasers in their respective paths and fine alignment includes translation of the focusing optics for each path as well as translation of the objective.

Course Alignment should be done with the Coherent 621 Laser run in Standby mode (i.e. the lowest power). However, when running a sample through the CyAn and searching for peak fluorescence it may be necessary to restore laser power to minimum regulated output.

ADP Rough Alignment without O2 Optics:

1. Alignment of the 488 Path: work from the source to the inspection point
   a. The First Kinematic Stage in the Prism Tower (MLE only)
      i. Check that the first 9882 is parallel to the laser beam- align if necessary by rotating complete 9882 mount
      ii. Loosen the mounted prism and rotate so that the back reflection of the laser ***is not*** going directly back into the laser head
      iii. Check that the laser is entering and exiting the center of the prism- translate stage if necessary (using all 3 adjustment screws- be careful to maintain top plane of prism parallel to beam).
      iv. Steer the beam to enter the center of the first prism of the "second prism set."

   b. The second stage in the Prism Tower (MLE only)
      i. Check that the second stage is parallel to the laser beam- align if necessary by rotating the entire stage.
      ii. Loosen the mounted prism and rotate so that the back reflection of the laser beam ***is*** going directly back to the center of the first prism
      iii. Iteration may be necessary between steps a.iv –b.ii. to ensure the light is entering and exiting the "second prism set"

c. Steering the 488/351 Beam
    i. Use blank beam-expansion mounts with Scotch tape, for targeting, placed over the oval beam window to steer the 488 beam down the center of the 488 path. Steer the beam by adjusting the horizontal and vertical screws on the second stage to steer the beam. Since the beam is being translated using the two prism elements in the "second prism set," the vertical adjustment knob will move the beam diagonally.
    ii. Remove 488, 351 focusing optics and FSC Detector assembly from the Inspection Block (claw). Rotate FSC bar to be "on edge" (i.e. blocking the smallest amount of light when viewed from the 488 path).
    iii. Once through the centers of both of the blank 488 beam-expansion mounts, adjust the 488 mirror to steer the 488 beam through the center of the 488 lens mount and hitting the center of the FSC obscuration bar (when turned on edge).
    iv. If a Cuvette is already installed rotate the Cuvette until the back-reflection is directly in line with the incoming light. The rotation is most exaggerated when looking at the exit point of the "second prism" set.
    v. Replace the 488 Beam Expander mounts with mounts that contain optics and replace the 488 focusing lens into the Inspection Block.
    vi. Run Leak-Tec (or some particle that is excited by 488) at a fairly high event rate and move the Cuvette in the forward/back direction until the fluorescence as seen by the human eye is brightest.
    vii. Using the Pinhole Camera and the sample channel images, center the Flow Cell left/right.

Similar to the MoFlo, once the 488 path is coarsely aligned, the position of the Flow Cell and Prism Tower should not be adjusted until fine alignment is done using calibration particles.

2. Alignment of the UV Path: work from the source to the inspection point (starting at the first stage on the optical table).
    a. The First stage in the UV Path on the Optical Platform
        i. Position the reflected UV portion of the 488/351 beam to be in the center of the first UV mirror by translating the stage.
        ii. Steer the UV beam from the first UV mirror onto the center of the second UV mirror.
        iii. The brighter of the two spots should go through the open hole in the special filter.

b. The Second 9882 in the UV Path on the Optical Platform
    i. Translate the second UV mirror to steer the beam through the center of the 351 lens mount and the Cuvette sample channel.
    ii. Adjust the vertical position of the beam on the cuvette sample channel to be slightly above the 488 path intersection on the cuvette.
    iii. Replace the 351 focusing lens into the Inspection Block.
    iv. Run Leak-Tec (or some particle that is excited by 351) at a fairly high event rate and adjust the horizontal and vertical screws on the second UV mirror until the fluorescence as seen by the human eye is brightest.

*Because there are two mirrors in the UV path, iteration between steps ii and iii may be required to center the beam on both mirrors.

Alignment of the 635 Path:

i. With a screen in place, rotate the diode laser until the elliptical beam is vertically orientated and tighten the set-screw. Remove the screen.
ii. While watching the cuvette sample channel, steer the horizontal and vertical adjustments on the laser stage to hit the cuvette sample channel.
iii. Adjust the vertical position of the beam on the cuvette sample channel to be slightly above the 488 path intersection on the cuvette.
iv. Run Dako cleaning solution (or some particle that is excited by 635) at a fairly high event rate and adjust the horizontal and vertical screws on the stage until the fluorescence as seen from the pinhole camera brightest.

Since red fluorescence is approaching the upper end of the visible light range, there will be only a small difference in fluorescence intensity when trying to "peak" the signal in step c. Therefore, you might need to watch the pinhole camera image while adjusting the horizontal and vertical screws on the stage.

CyAn Fine Alignment

These steps hold true weather the system does or does not have the O2 Optics.

1. Turn on CyAn according to the CyAn Quick Start Guide instructions and give at least 30 minutes to warm up for non UV options and one hour for UV option.
2. Disable the Laser interlock on the CyAn.

3. Be sure 488 beam is centered horizontally in the lens (#1) just before the directional mirror. If it is not centered on the MLE go on to the next step. If it is centered, proceed to step 5.
4. To center the beam on the MLE
   a) Remove the back cover. (have someone help)
   b) Locate the stage at the middle, bottom of prism tower
   c) There are three set screws on the stage. Do not adjust the pivot point set screw
   d) The set screw located against the prism tower that is not the pivot point can be used to adjust the beam if it is not centered vertically

   e)The set screw located away from the prism tower can be used to adjust the beam if it is not centered horizontally

5. Open the following histograms in Summit if necessary
   a. FSC v SSC
   b. FL1-FL9 (single parameter- all set to Lin/Peak)
6. Mix SpectrAlign beads to reach a concentration of approximately 1million per ml. (About five drops of SpectrAlign beads into approximately 1 ml of DI .)
7. Run beads on CyAn around 100 eps and check that the CVs are with in specifications by placing bar regions over each peak. If necessary adjust PMT voltages/gains to bring peaks on scale. Gate all of these histograms on the main scatter population in the FSC v. SSC histogram. (see attachment for specifications)
8. If the CVs are not within specification or you have no signal, first be sure there is no air in the flow cell. Run several debbuble cycles and run 70% ethanol on high (be sure to flush out completely).

Note If CVs are still not with in specifications, visually inspect the cuvette for bubbles. It is very common for a bubble to cause poor CVs. If bubbles are present use the debubble button while watching cuvette to see that they are removed. If removing the bubbles does not help the CV's alignment is required.

9. Determine at this point if rough alignment is needed or fine tuning. If you see no signal in your plots and have no event rate you must rough align the 488 beam. If you have a population, but your CV's are not within specification, you only need to fine-tune the alignment.
10. Create a FL1 vs FL4 histogram.
11. Activate Cycle mode in Summit with a refresh rate of 100eps.
12. Acquire data around a rate of 200 eps.
13. For rough alignment, check where the 488 beam is hitting the obscuration bar (#2). If it is not hitting the center (horizontally), then the mirror (#3) needs to be translated over. It is very important that the beam is centered horizontally on the obscuration bar (#2) and the lens (#11).
14. To translate the 488 beam over, turn each screw (vertical, horizontal, and pivot- see figure C-on the #3 mirror) in the direction the beam needs to go

in order to be centered on the obscuration bar. Repeat steps 12-14 until beam is centered.

15. Once the beam is centered on the obscuration bar move on to fine alignment. On the 488 mirror (#3) adjust the horizontal and vertical screws. **(NOTE: Do not adjust the "pivot point" screw- see attached diagram.)** Watch the data in Summit and see if the adjustments either improve or degrade the CVs. When aligning be sure that each screw adjustment is an improvement before moving to the next screw.
16. Every now and then bring the event rate to 100 eps and check the CVs of the beads. Be patient, it can take several iterations of adjustments to bring the CyAn into optimal alignment.
17. If the CVs are still not within specifications, continue alignment with the flow cell.
18. If adjusting the mirror did not help enough, then adjust the flow cell (#4) by turning first the screw to the right of the flow cell to maximize signal, then the screw on the front of the flow cell. Touch up the alignment with the 488 mirror (#3) after adjusting the flow cell. Again watch in Summit to see how the adjustment affects the data.

NOTE: **This should only be a small turn for either screw, perhaps 1/50 of turn at most. Moving the flow cell too much will require the adjustment of the beam focusing optic and the objective.** This step may have to be repeated several times to reach the specification. Be patient. If the CVs are still not within specifications, proceed to filter alignment.

19. The dichroic filters can be adjusted as well. They have a dowel to hold them in place, but there is some play to this. Just remember that as you adjust a dichroic to optimize one channel, it can adversely affect the channel to its opposite.
20. Once the 488 beam is aligned, then move to the red laser alignment (#5). Change the axis on the FL1 vs FL4 histogram to FL8 and FL9.
21. If there is no signal present, try adjusting the laser delay in the Control Panel either up or down. As you adjust the laser delay, watch the data in Summit and see where the data falls both at the high and low points. Make a note of where the mid point between the high and low values is and leave this as the laser delay value.
22. For the laser adjustments, adjust the horizontal and vertical screws on the diode laser (#5) as needed. **DO NOT move the flow cell or adjust the pivot point or any 488 mirror adjustments.**
23. Again dichroic filters can be adjusted, but this can affect other channels.
24. Once the red laser is aligned, move to the third laser (UV, violet) mirror (#6).
25. Change the FL8 vs FL9 histogram to FL6 and FL7.
26. Adjust the horizontal and vertical screws on the mirror as needed (#6). **DO NOT adjust the flow cell or pivot point or any 488 mirror adjustments.** Check to see that the CVs meet specifications.

28. If by adjusting the mirrors and dichroics the system cannot be aligned there can be a couple of other options for alignment.
29. The position of the first dichroic is very important. It is good to check that the collected light is going down the optical pathway correctly.
31. Remove all dichroic mirrors except the first one (#7). Be sure to put the mirrors in a clean, protected area.
32. Run a totally empty tube on CyAn.
33. Note the frosted “window” (#8) at the end of the PMT block. There are several cross hairs in the window.
33. Spots from the emitted light should appear in the window.
34. Move the first dichroic (#7) so the spots are centered on the cross hairs. It is okay if the spots are not centered vertically, but they should be centered horizontally.
35. Place the second dichroic in place. Adjust the second dichroic so the spots are centered horizontally.
36. Place each of the remaining dichroics in one at a time, adjusting each so the spots are centered horizontally. (The spots will become dimmer after replacing multiple dichroics.)
37. If the system is still not within specifications, call Dako Support for further steps.

Figure 5.1: CyAn ADP

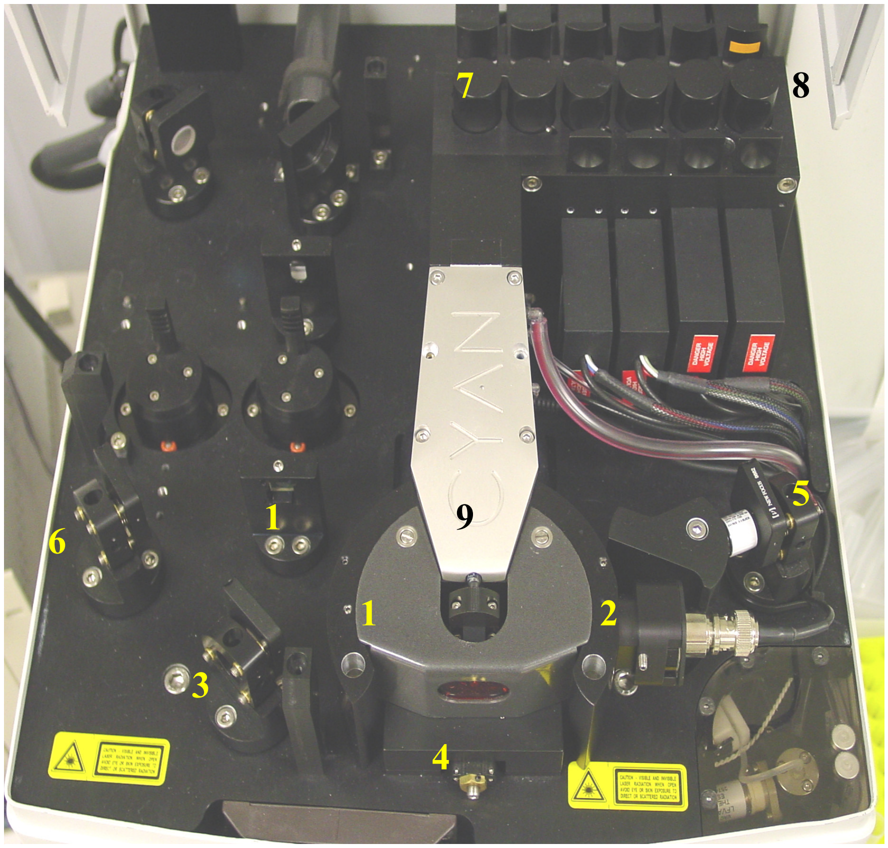


Figure 5.2 CyAn ADP UV Model

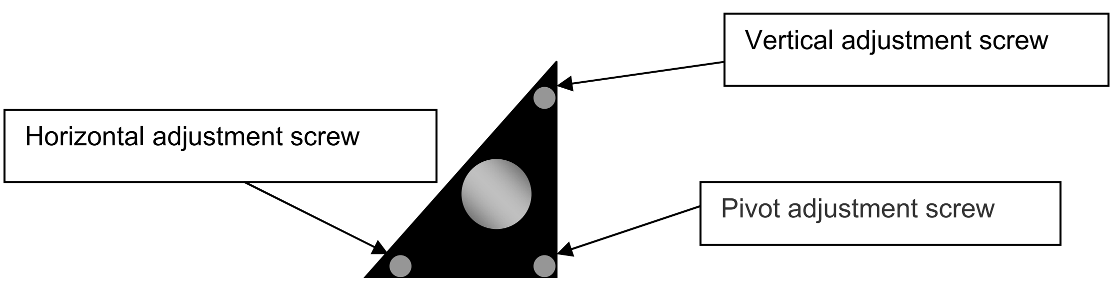
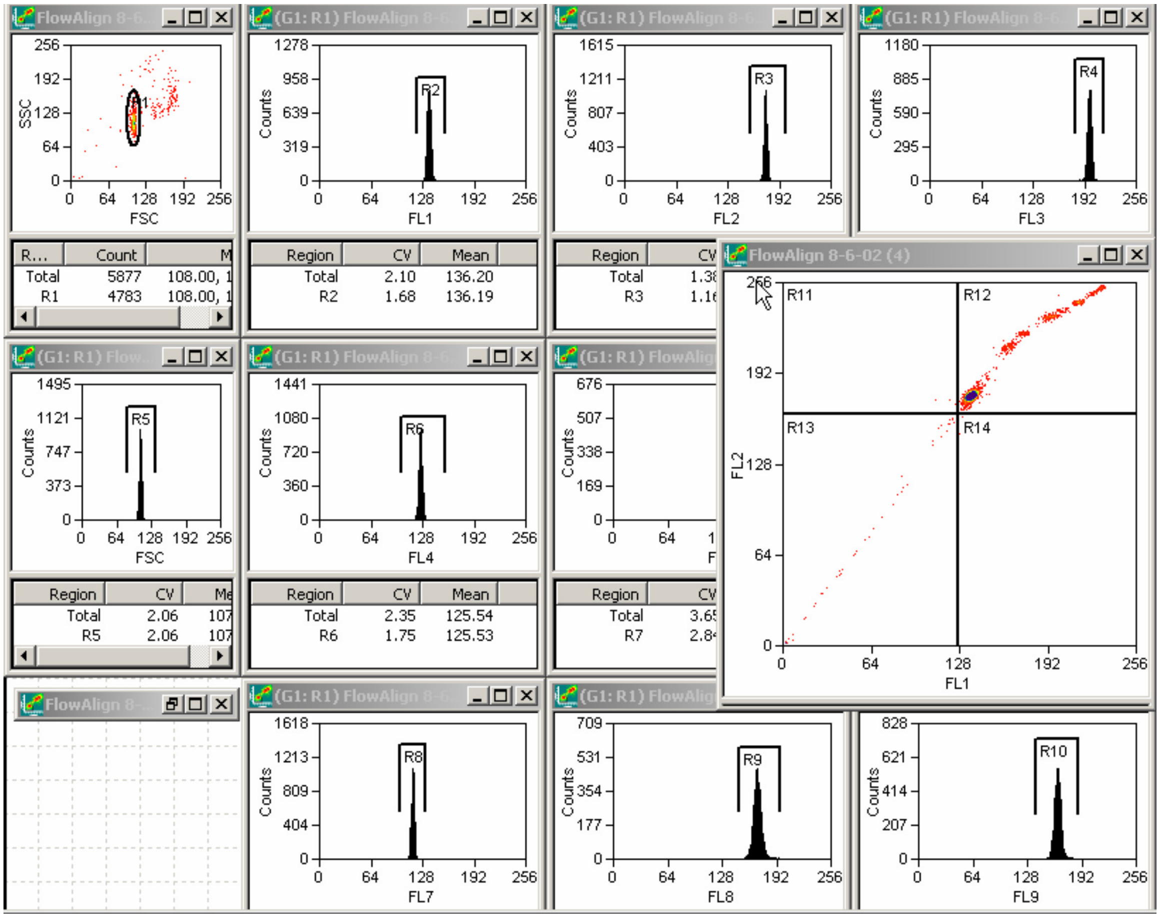


Figure 5.3: Alignment stage diagram



Items to remember relating to alignment

Cycle Mode

Cycle Mode in Summit will cycle the events through a buffer to display only the most recent data events. The number of data events displayed at any one time is adjustable. Simply click on the Acquire pull-down menu and select Cycle Amount. This adjustment can only be done while not acquiring. Usually it is best to display from 100 to 200. This way as the sample is displayed any changes in alignment can immediately be seen.

Gating

Gating on the main population in the FSC vs. SSC histogram will clean up the data and %CV values in other histograms. This eliminates bead or cell fragments and doublets from being considered in the alignment of fluorescent channels.

Align in Linear Mode

Aligning in Linear Mode on the ADC's will provide better sensitivity to changes in the alignment. Better sensitivity to alignment will allow for the fine-tuning of the mirrors. Do this by right-clicking on the axis and select or de-select the Log Parameter.

The Pressure Differential

In general, the Sample Pressure should be approximately 0.1-0.3 psi greater than the Sheath Pressure. This is known as the Pressure Differential. The CyAn runs at a sheath pressure of approximately 4.8 psi at the flow cell. This means the sample pressure should be no greater than 5.1 psi.

Driving Too Fast

The CyAn is designed to process events very, very quickly. In fact, precise results and data can be achieved at speeds in excess of 50,000 events per second. However, driving this fast carries with it some responsibilities. Namely, as the driver, you need to make sure that your cell concentration is such that you can achieve high event rates at an acceptable Pressure Differential.

Remember that the CyAn electronics prefers to process cells arriving at the interrogation point in single file. If you increase the Pressure Differential too much (>0.5 psi), this will cause the cells to arrive at the interrogation point simultaneously and will therefore increase coincidence.

In summary, if you wish to process cells at a high event rate, make sure your sample is adequately concentrated.

General Rule for bead and cell Concentration:

For every 1,000 events per second that you wish to obtain, you should have 1 million cells per milliliter. For example, if you wish to run at 40,000 events/second, you should have at least 40 million cells/milliliter. This will ensure that you can obtain a high event rate at an acceptable pressure differential.

Alignment Goals

Increase Intensity

The main goal of alignment is to increase the intensity of a given population until it is maximized. This means pushing the population in a single parameter histogram to the right as far as possible. The greater you maximize the intensity of light observed by a detector, the better aligned the system will become.

Reduce %CV Value

Another equally important goal of alignment is to minimize the % CV value for a given population. The % CV, percent coefficient of variation, is the standard deviation of the population, divided by the mean, times 100%. This is a judgment of the width of the peak or the spread of the population. Small % CV values are important and often necessary to resolve neighboring populations.

Inter-Laser Delay

The three laser interrogation points on the CyAn are separated in three different planes with the 488 laser interrogation point on bottom, the UV/Violet in the middle and the Red on the top. As a particle is aspirated upward in the sheath stream, it passes through these interrogation points at three different points in time. The Inter-Laser Delay is defined by the amount of time it takes for a particle to travel from the primary laser interrogation point to the secondary laser interrogation point. If it takes, for example, 1.6 ms for a cell to travel from the primary interrogation point to the second, it will take approximately another 1.6 ms to travel from the second to the third laser interrogation point.
The Sheath Pressure is the only variable that will affect the stream velocity and therefore warrant a change in the Inter-Laser Delay value.

Determining the Inter-Laser Delay

The Inter-Laser Delay value is a time constant that is characterized by the velocity with which the particles are traveling in the stream. This time constant defines the time that it takes for a cell or particle to travel from the interrogation point of the 488 laser to the interrogation point of the UV/Violet laser, and in turn from the UV/Violet to the Red laser.

This value can be changed in two places in summit software. It can be changed in the Edit Preferences section or by pressing **CTRL+ALT+F1** at the same time a special window will appear. The second option will allow for changing the delay as the acquisition occurs.

Laser Delay Optimization: To be performed on both path 2 and path 3

1-With Summit open, press a combination of CTRL, ALT, and F1. This will open a dialog box where one can adjust the laser delay of path 2 violet channels and path 3 red channels.

2-Put both of the two channels from each path into Linear Area parameter displayed on a single parameter histogram. Re-apply gate from FSC vs. SSC histogram.

3-Place Summit in Cycle display mode Cycle Count- 100 events and set the event rate to ~100 events per second.

4-While triggering on FSC increase the threshold until your population of interest begins to be clipped. Lower the threshold to just below clipping the population of interest. Note- the median of the Area parameter will decrease with an increase in threshold,

5-Adjust the relevant path's laser delay to find the highest median value. Next adjust the laser delay in one direction until both the median and cv begin to degrade. Record this value. Then adjust the same laser delay in the other direction until the median and cv degrade by a similar amount. Record this value. Set the laser delay to the average of these two values.

6-Repeat steps for the other path and close laser delay adjustment window.

As a general rule, when running at 4.8 psi on the CyAn ADP the delay values are around 25 for path two (UV/violet) and 50 for path three (red).

Section 6

Technical Aspects

Threshold

The purpose of the threshold is to desensitize the electronics to low-level noise caused by very small particles or auto-fluorescence from the data.
The Threshold Level adjustment is located in the Control Panel in Summit software.
It's important to keep the threshold as low as possible to guard against losing valuable or meaningful data.

Delay between Lasers

The Inter-laser delay setting will assist the electronics in the timing of a particle from the first Laser/Pinhole to the second Laser/Pinhole and in turn to the third.

Each ADC module is set to activate at the appropriate time, which is determined by the setting of this delay.

Lasers and Optics

CyAn Optical Components

Lasers
Steering Optics
Optical Table
Filters and Fluorescence
635 Diode Laser
Beam Shaping Optics
Sensitivity and Spectroscopy

The Laser

Basic Laser Theory

The theory of operation is nearly identical for all water and air cooled lasers with the exception of tunable lasers. The tunable lasers will house an adjustable prism, which can be tuned by the operator to alter the monochromatic output to a variety of emission lines.

In the water and air cooled models, the electrical discharge in the laser creates a plasma in the glass enclosure where the atoms in the plasma will have a high percentage in the electronically excited state as opposed to the ground state. This is known as a population inversion and is required for stimulated emission. Stimulated emission produces monochromatic and coherent light that is bounced back and forth between the High Reflector and the Output Coupler mirrors. The High Reflector mirror is designed to reflect as much light as possible, while the Output Coupler mirror will transmit a small fraction of the coherent light and reflect the rest.
The gas reservoir in the laser cavity will hold a variety of gases depending on the type of laser and its output characteristics.

The laser cavity: The cavity defines a unique axis with very high optical gain beam direction.

Light bounces back and forth between mirrors gaining in intensity with each pass though gain medium.
Some light escapes through output coupler and laser reaches stable equilibrium.

Figure 6.1 shows some of the typical components of a laser.

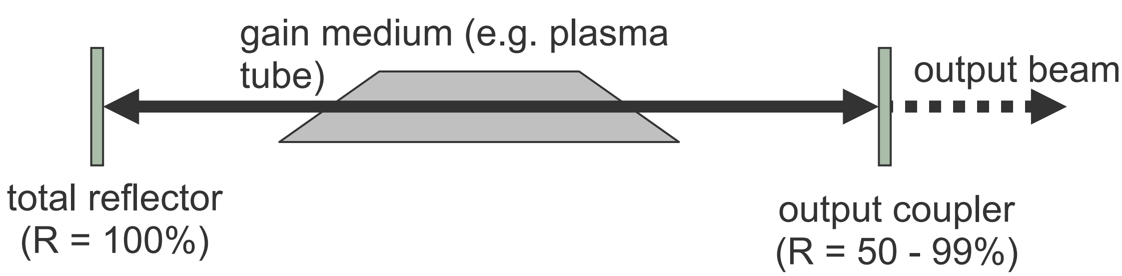


Figure 6.1: Typical Laser Components

Figure 6.2 shows the common laser lines used in flow cytometry.

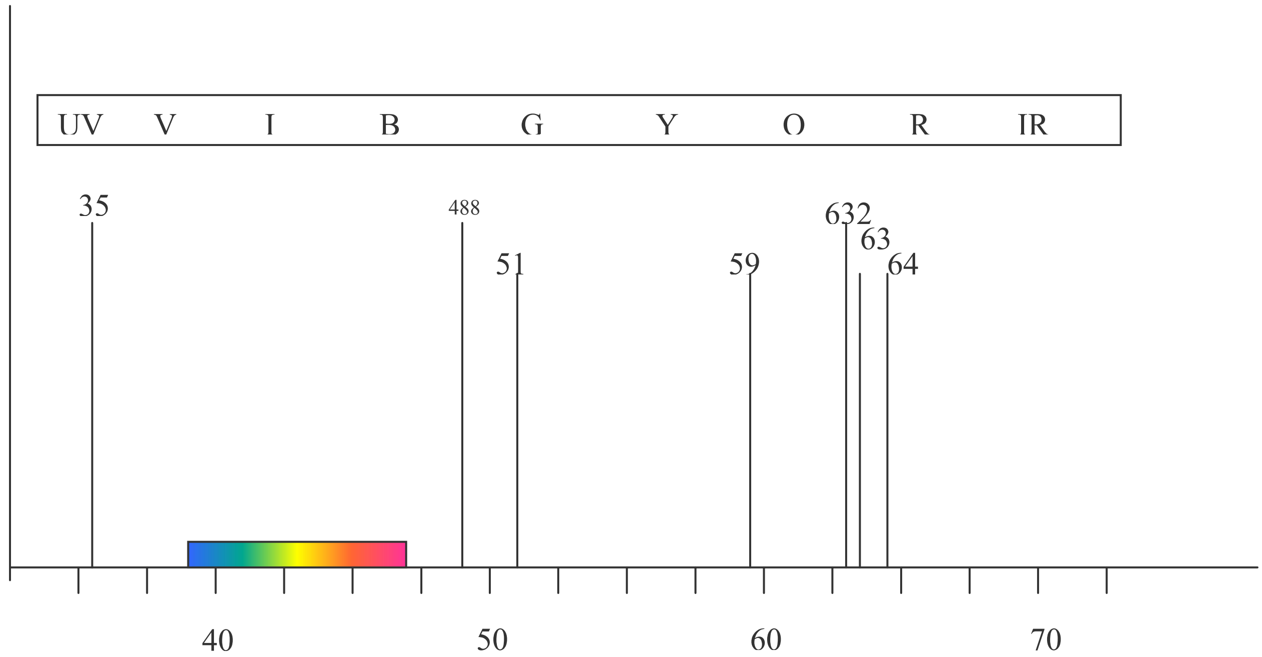


Figure 6.2

Diode Lasers

Diode lasers offer several advantages over traditional gas lasers. They are much smaller, require less power, have greater efficiency, and don't require cooling. These are all very compelling advantages and many feel diode lasers will play a very important role in the future of flow cytometry. The current disadvantage is the power capability of current diodes.

Multiple Line Operation

To sustain gain as light travels back and forth within the laser, the waves must remain in phase, i.e., the round trip from output coupler to high reflector must be an exact multiple of the wavelength. Several different wavelengths can satisfy this requirement and contribute to the emission of multiple emission lines from a single laser. It is sometimes desirable to have multiple line emission and in fact, an argon laser tuned for UV emission is always in multiple line mode. Laser selection and tuning should always be respective of the application. For example, an emission from the laser should not impede with the bandpass filter channel of a fluorochrome of interest.

TEM Modes

The ideal beam has a Gaussian cross-section and is called TEM00 output mode. The energy distribution of the TEM00 mode is conical in nature with the highest intensity of the beam being in the center of the cross section. The intensity will drop off gradually to the outer ring.

Other TEM modes are more prevalent when using a more open aperture on the laser. Ideally, the laser aperture should be set between 60 to 80% of the maximum beam diameter (wide open is usually 0 or zero) to ensure the best energy distribution with least laser contamination of adjacent detector paths.

Troubleshooting the TEM Mode
When a TEM mode other than TEM_{00} is being produced by a laser, this means that a cell passing through the beam profile can generate a non-Gaussian analog output from the detector because of the discontinuities in the beam profile. This can cause increased hard coincidence aborts and degrade the CV's of your standard calibration particle and cells.

Determining the TEM mode can be difficult and dangerous, because it usually requires deflecting the laser beam over a large distance to magnify the imperfection. A device called a Mode Cup can be used to help direct the beam to a distant surface. If you are not certified to work on a laser or do not have the proper personal protective equipment, do not attempt to work with the laser. Call the Service Department at Dako for support.

Beam Shaping

Beam Shaping Optics

The CyAn has beam shaping optics placed inside of the claw that bring the beam to focus at the flow cell. The beams of the lasers should pass in the middle or close to the middle of these optics in order to be in the correct position at the flow cell.

Benefits of Beam Shaping Optics

The elliptical beam profile provides several benefits to enhance performance. First of all, the flattened slit-like profile provides a shorter and more intense beam of light through which the cells will pass. This will ensure that a cell will spend less time in a more intense beam, which reduces the pulse width and will enhance doublet discrimination.

The flattened beam profile will also reduce inter-laser cross talk and enhance CV's, particularly when operating at high event rates and high pressure differentials.

Optical Filters

Optical filters are designed to block, pass, or reflect light of certain bandwidths and in the case of the dichroic filter, reflect and pass light of different wavelengths at the same time. Filters are either made from dyed glass, which will absorb certain wavelengths of light, or dielectric coatings that have been vapor deposited on a glass substrate. The coated filters function by internal reflection and interference between the dielectric deposition layers. The list below describes the features of some commonly used filters in flow cytometry.

Bandpass Filters transmit light only within a defined spectral band ranging from less than one to many nanometers wide.

Longpass and Shortpass Filters transmit only above or below a certain "cut-on" or "cut-off" wavelength and continue to transmit a wide energy band.

Dichroic Beamsplitters are used at a non-normal angle (usually 45^{o}). The longpass and shortpass dichroic filters are designed for optimal reflection of one specified region of the spectrum and high transmission of another.

Neutral Density Filters will uniformly attenuate the intensity of light over a broad spectral range.

Rejection Band Filters are designed to block a narrow spectral band, such as a monochromatic light from a laser, while transmitting other wavelengths efficiently.

Filter	Characteristic	Example
Bandpass	Passes a selected band of wavelengths	570/40BP
Longpass	Passes longer wavelengths than the cutoff	740LP
Shortpass	Passes shorter wavelengths than the cutoff	650SP
Dichroic	Shortpass or longpass. Defined by a cutoff wavelength.Reflects at 90° what it doesn't pass.	605DCSP or 605 DSP
Neutral Density	The ND number represents the amount of attenuation on a log scale. A 0.3 ND will reduce the transmitted light by ½. A 1.0 ND filter will attenuate 10x the light intensity (reduce to 1/10th).	0.6ND
Reject	Blocks a selected narrow band of wavelengths.	488RB

Choosing Filter Sets

Considerations should be given to the type and number of fluorochromes to be used during a multicolor application. If you are unfamiliar with the spectral emission of a fluorochrome, this information can usually be obtained from the manufacturer of the monoclonal antibody. It is important to be familiar with the spectral distribution of each fluorochrome, and the potential for emitting wavelengths that will enter a neighboring bandpass filter. Such spectral overlap can be handled using compensation, however, it's always a good idea to minimize this overlap by selecting filters that will reduce the need for large amounts of compensation.

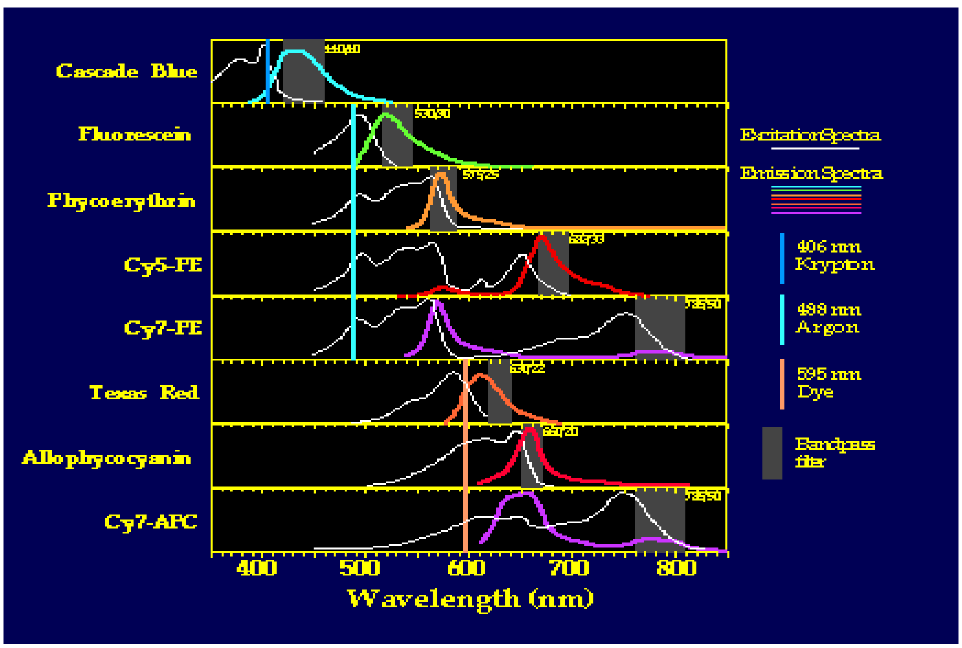


Figure 6.3: Spectra of some commonly used dyes

Preventing Laser Crosstalk

Another consideration should be the lasers in use on the CyAn and the possibility of laser crosstalk. Laser crosstalk is defined as the emission of one laser line being transmitted into another laser's pinhole. In this instance, laser light can produce a level of background noise in an unexpected bandpass filter. Crosstalk can be eliminated by selecting the appropriate dichroic, bandpass or reject band to block the wavelengths of the other two lasers.

Positioning the Filters

Notice in the outlay of the filters, that the longer wavelength bands are directed to their detectors by passing through the fewest possible filters. This is because filters themselves can absorb/reflect as much as 20% of the light passing through its glass. Longer wavelength bands are lower in energy and are most susceptible to this loss in intensity.

Aligning the filters

When aligning the filters, you should align the number one dichroic first. Run a bright fluorescing bead or fluid for 488 excitation and note the emission spot at the cross hatch at the end of the PMT block. The emission spot should be in the middle of the cross hatch. Adjust the first dichroic appropriately until it is centered on the top cross hatch. Then, introduce dichroic filters one at a time and adjust filters so emission spot is centered on the cross hatch each time.

Installing and cleaning filters

To replace a bandpass or dichroic filter, just pull the holder out of the PMT block and replace with new filter inside of a new filter holder. If need be a dichroic filter may be removed from a filter holder, however they are glued in with RTV. The RTV must be completely removed from the holder in order to ensure the new filter will lay flat in the holder. Bandpass holders are held in place by a set screw and can be removed by loosening the set screw. Always wear powder-free latex gloves when handling filters to prevent fingerprints from harming the filters.

Photo Multiplier Tubes (PMT)

The PMTs on the CyAn are manufactured by Hamamatsu. There are up to 10 total. There is one for Side scatter and one for each fluorescent channel. They each have a signal cable that ends in a female, gold SMB connection. The signals from these go into the preamplifier boards in the PXI chassis.

Section 7

Troubleshooting

The basic philosophy for troubleshooting should be to concentrate on how the CyAn is supposed to work first and what good data looks like. By learning the signal pathways as well as the installation procedures and testing, troubleshooting becomes a much easier task. This section is intended to be a guide for common situations.

Fluidic System

Common Fluidic Issues

Instability

Many internal and external factors can produce system instability and can be difficult to isolate. On MLE models the **LP5 heat exchanger** that cools the 621 laser is commonly a culprit. Always inspect the fluidic **umbilical** to be sure it is not being vibrated, bumped, or pinched. It is always a good idea to treat a system experiencing some form of fluidic instability with a **warm solution of Triton X detergent** and DI water as outlined in the "Air Bubbles" section of this guide.

To isolate fluidic instability from laser instability run the stability test from the Cytometrics test procedure and compare the data to the examples below to try to evaluate the type of problem you are experiencing. Once the type of instability has been deduced, move on to the next appropriate section of the trouble shooting guide.

Result set 1	Result set 2	Result set 3
Median values gradually rise or fall together across all fluorescence channels spanning all 3 (or 2) laser paths	Median values spike or fall suddenly and recover on all or most fluorescence channels across multiple laser paths	Median values across channels residing on a single laser path rise or fall gradually together. Or data is inconsistent across laser paths.
FLUIDIC INSTABILITY	**FLUIDIC INSTABILITY**	**LASER INSTABILITY**
Unstable sheath pressure or unstable core stream	Air bubbles	Laser power fluctuation

Air Bubbles

If air bubbles are visible begin tracing the system from the flow cell to the SMS. Inspect all fittings for tightness and that all have been installed with thread sealer. Check all hose connections for damage, wear, or a loose barb fitting. Inspect plastic barbs for score marks that may have been left by a razor knife cutting away old tubing.

If bubbles persist clean the system with a solution of 1ml Triton X detergent and 1 Liter of DI water. If possible, heat the DI water to near boiling before adding the detergent. Run the entire liter of detergent solution through the system and flush with 1 Liter of clean DI water.

Unstable Sheath Pressure

Tee into the hose running from the "GA" port of the **system regulator** and connect to a digital pressure gauge. Run the system and watch the readout on the gauge. If the pressure fluctuates more than +/- 0.3 PSI, verify that the system regulator is calibrated correctly. If the fluctuation persists replace the system regulator. If the system pressure is stable continue to the next paragraph.

Tee into the hose running from the "GA" port of the **cleaner regulator** and connect to a digital pressure gauge. Run the system and watch the readout on the gauge. If the pressure fluctuates more than +/- 0.3 PSI, verify that the system regulator is calibrated correctly. If the fluctuation persists replace the system regulator. If the system pressure is stable continue to the next paragraph.

Tee into the hose running from the "GA" port of the **sheath regulator** and connect to a digital pressure gauge. Run the system and watch the readout on the gauge. If the pressure fluctuates more than +/- 0.1 PSI, verify that the sheath regulator is calibrated correctly. If the fluctuation persists replace the sheath regulator.

Unstable Event Rate / Unstable Sample Pressure

Tee into the hose running from the "GA" port of the **system pressure regulator** and connect to a digital pressure gauge. Run the system and watch the readout on the gauge. If the pressure fluctuates more than +- .3 PSI, verify that the system pressure regulator is calibrated correctly. If the fluctuation persists, replace the system pressure regulator. If the system pressure is stable, continue to the next paragraph.

Tee into the hose running from the sample collar to fluidic manifold and connect to a digital pressure gauge. Run the system with a sample running and watch the readout on the gauge. If the pressure fluctuates more than +- .05 PSI, verify that fittings are tight and o-rings are intact and undamaged. If the fluctuation persists replace the sample pressure regulator.

Unstable Core Stream

An unstable core stream can usually be diagnosed by connecting a monitor to the pinhole camera and running a sample of DI water with 1 drop of Fluorescine (Leak-Tek). As the sample pressure is increased the visible fluorescence spot on the monitor should grow larger and brighter, but should not drift to the left or right. If the stream is drifting to the side, try adjusting the height of the injection needle inside the flow cell and check the centricity of the needle in the flow cell cone. If the needle is bent or otherwise damaged replace the needle and the black two-piece compression nut that secures the needle in the flow cell.

Section 8

Technical Procedures

How to install a CyAn

Please refer to the CyAn Installation Technical Procedure (Doc No. 0000129) and CyAn Installation Form (Doc No. 0000100) for information on CyAn installation.

Sheath System Decontamination

Please refer to Cyan ADP Sheath Decontamination Technical Procedure (Document number 0001104).

Tube Present Sensor Calibration

The Tube Present Sensor (TPS) may require cleaning, repositioning or calibration in order to correctly and reliably identify the presence of a test tube. There is also another consideration.

Gray colored mounting-plates (Plate Sample Collar), if present on the instrument, should be replaced with the brighter, non-anodized version to increase the voltage span produced from the optical circuit. Calibration and alignment will be easier and much more predictable.

Tools:

Jeweler's screwdriver (suitable for adjusting a potentiometer)
Voltmeter
Hex wrenches, metric

Time required:

1/2 hour.

Parts, as needed:

996313- Spare, Plate Sample Collar, CyAn ADP.

Mounting Plate

An unknown quantity of instruments have been assembled using mounting plates that are less than ideal with respect to the amount of reflected light they provide for the Tube Present Sensor (TPS). One version of the plate, coated Gray, makes alignment of the TPS much more difficult than otherwise expected.

A new mounting plate (996313- Spare, Plate Sample Collar) should be installed, when Gray mounting plates are encountered before attempting any alignments of the TPS. Go to Electronic Adjustment if the instrument already features this brighter plate (see photo 8.1).

1. Remove the outer cover and free the fluidics module from the instrument chassis by removing the two-screws shown in photo 8.2.

2. You may now pull the assembly slightly forward and away from the instrument; beware of cable and tubing lengths.

3. Remove Plexiglas from front right corner of optical top plate by removing the four screws holding it in place.

4. Remove sample tubing from pinch valve and Tube within a tube assembly.

5. Remove the Tube within a tube from the mounting plate (three-screws) along with the waste and sample air tubing. (see photo 8.3)

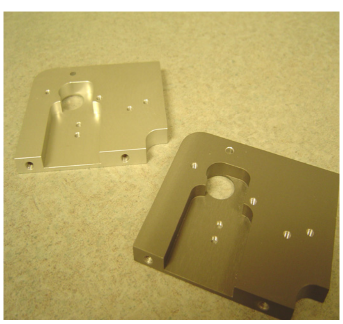


Photo 8.1: *Bright & Grey plates.*

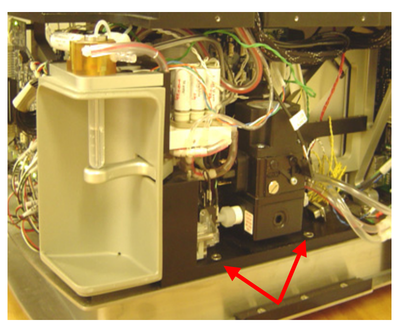


Photo 8.2: Unbolt Fluidics module

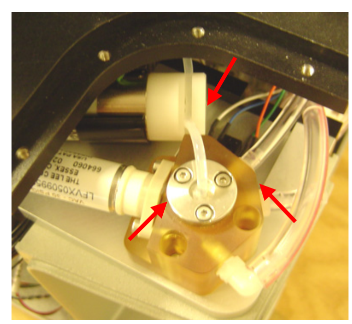


Photo 8.3: Remove Tube in a tube.

6. Exchange the mounting plate for the bright, aluminum type (photo 8.1):

   - Remove the pinch valve and its bracket from the mounting plate.

   - Remove the old plate (there are four-screws) and install the new mounting plate.

- Install the Tube within a tube and reconnect the waste and sample air tubing.

7. Mount the fluidics assembly into the instrument before continuing to Electronic Adjustment.

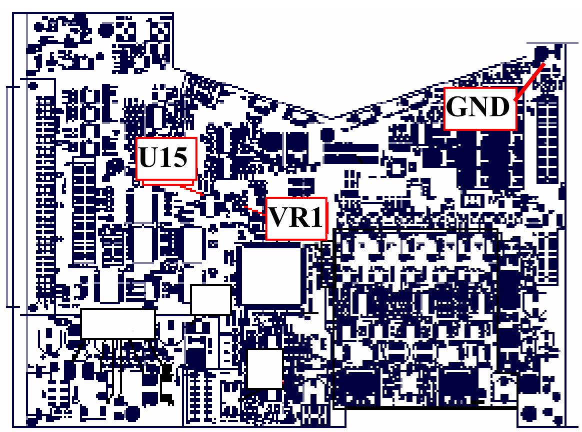
Electronic Adjustment

The tube sensor circuit must provide, approximately, a 6 Volt or greater potential between tube-in and tube-out states; centered at 0 Volts. However, the tube-out voltages supplied by the circuit should not exceed a positive or a negative 9.9V.

NOTE: strong ambient lighting will prohibit or interfere with electronic alignments.

Allow the instrument to warm up for at least 15 minutes after starting Summit Software.

Insert a test tube into the instrument and measure and record the voltage between U15, pin1 and GND on the main electronics PCB, remove the test tube and record the voltage again.



***Drawing 8.4**: CyAn's main circuit-board test points.*

Below are three-examples of what you may expect to find based on the voltage measurement acquired in Step 2 above: *Voltage too high*, *Narrow Range* and *Voltages not centered.* Follow the instructions described in the examples below that best describes your instrument.
NOTE: rotating VR1 clockwise will lower a voltage reading; counter clockwise will increase it.

Example 1: Voltage too high

Measured voltages, tube-out/tube-in: 10.5 V/3.3V

There is a satisfactory amount of difference between the two-conditions but the tube-out voltage is more than 9.9V.

Remove the tube and adjust VR1 until the reading is at, or slightly below, 9.9V.

Measure the tube-in and tube-out voltage to determine the average and adjust VR1 until the two-conditions are roughly centered about 0V.

Example 2: Narrow Range

Measured voltages, tube-out/tube-in: 1.1V/-1.2V

There is a compressed range of only 2.3Volts. The difference is so small that Summit may not be able to consistently distinguish if a tube is actually in or out of the instrument. Proceed on to Positional Adjustments to make position adjustments and then repeat Electronic Adjustments.

Example 3: Voltages not centered

Measured voltages, tube-out/tube-in: 8.5 V/1.3V

In this instance, you could expect to obtain voltage readings in the neighborhood of 3.6V with the test tube removed form the instrument and about -3.6V with the tube inserted. Adjust VR1 until the two-conditions are roughly centered about 0V.

Now check the test-tube icon in Summit. It should display the correct test tube status.

Remove the test tube, record the tube-out voltage and replace the instrument's cover (but do not yet affix it with screws)

Wait approximately 15 minutes for temperature equilibration inside of the CyAn. Remove the cover and immediately measure the tube-out voltage to be sure the values did not change substantially.

Do not proceed on to Position Adjustments if the instrument is now in working order.

Position Adjustments

If you are not able to obtain a satisfactory voltage difference between the tube-in and tube-out states then there is a misalignment between the optical sensor and the back wall of the tube within a tube mounting-plate. The sensor may require repositioning or even replacement.

Remove the cover above the Tube within a tube assembly for access to the TPS (photo 8.5).

NOTE: strong ambient lighting will prohibit or interfere with position adjustments.

Slowly move the sensor (photo 8.6), after loosening the 2mm setscrew, in/out and left/right until there is an acceptable change in voltage measurements between tube states. Be aware that the sensor's lead wires are easily damaged; they should not be pulled or unsupported.

Once you have a voltage difference of 6V or more, repeat the steps detailed in Electronic Adjustments. Only if there is difficulty in establishing a good voltage span should you:

Reposition the Tube within a tube (loosen the three-2.5mm screws). If this enables you to obtain a good voltage differential, repeat Electronic Adjustments.

a) Remove the TPS to examine the lens of the sensor and clean as needed.

Photo 8.5) *Remove the Tube within a tube cover.*

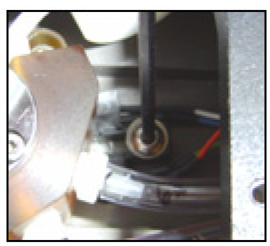


Photo 8.6) Move the TPS to improve voltage output.

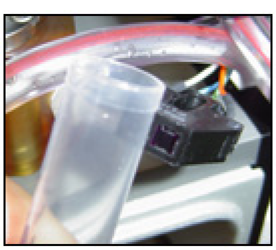


Photo 8.7) *Checking the operation of the TPS.*

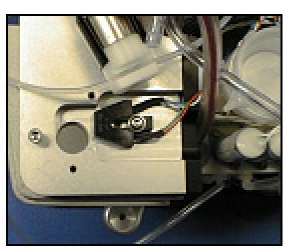


Photo 8.8) *The TPS is 1/16" from opening and off-center.*

b) Move an object in front of the TPS while looking for voltage changes at U15, pin1 (see photo 8.7) in excess of 5 to 6 Volts.

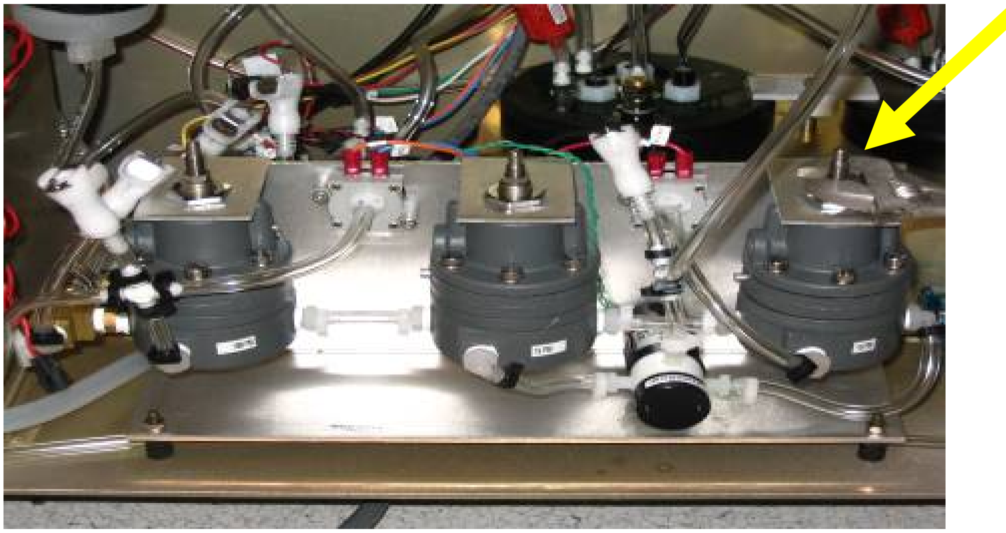
c) Reinstall the sensor with the printing facing up. Be certain that it does not block the tube hole (photo 8.8). Continue making adjustments until the voltage span is acceptable and then repeat Electronic Adjustments.

CyAn ADP Sheath Pressure Calibration

1. If fluidics are on, shut down fluidics.
2. Connect the **Inline Fluidics Test Cable ("T" connection)** with Cyan fluidics assembly if not already connected and connect the **Pressure Tester.**

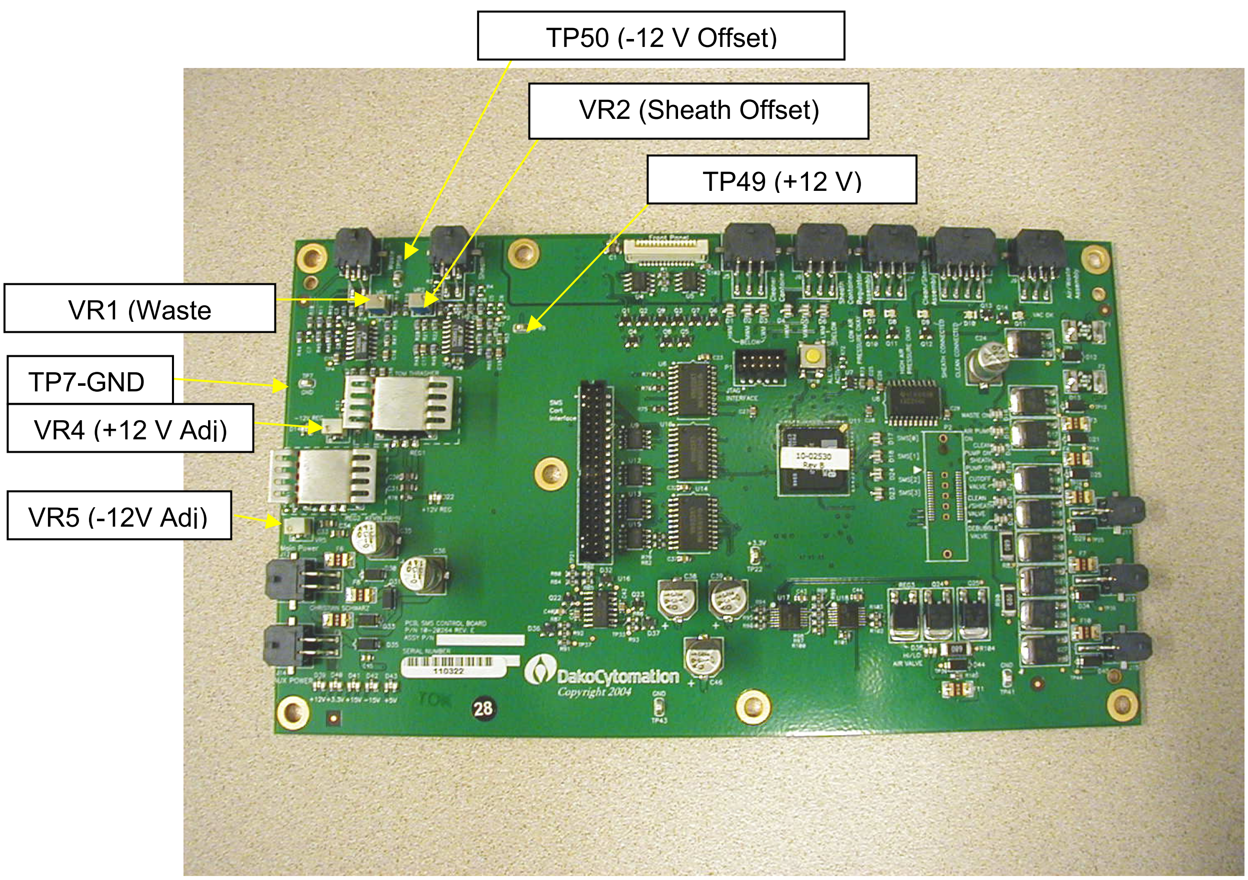
Pressure Tester

3. Turn on fluidic system of CyAn ADP.
4. Remove SMS rear panel if present and loosen lock nut on right pressure regulator

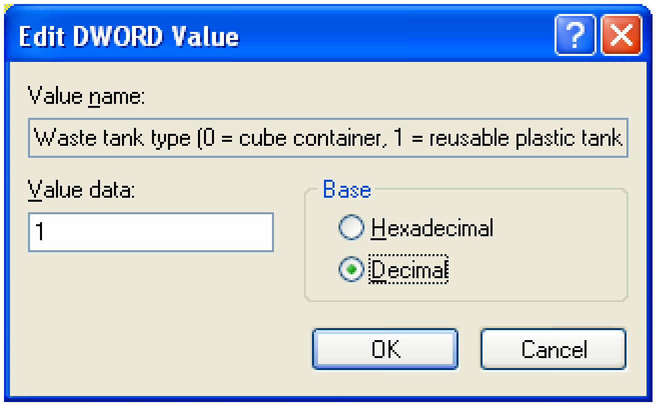


5. Adjust Sheath Pressure to 4.8 PSI using a flat head screw driver.
6. Tighten lock nut on right regulator, stop fluidics, and install SMS rear cover.
7. Turn off fluidics and remove “T” connection.

Sheath and Waste Load Cell Calibration



6. Connect voltmeter leads to the **TP 7 (GND Test Point)** and to **TP 49 (+ 12 V Test Point)** on the control board.
7. Adjust **VR4 (+12 V Adjust)** with the flat-blade screwdriver to set voltage to +12.0 +/- 0.1 V.
8. Touch positive voltmeter lead to **TP 50 (-12 V Test Point)**.
9. Adjust the **VR5 (-12 V Adjust)** potentiometer so that voltmeter measures -12.0 +/- 0.1 V
10. In the CyAn I\O click on the Analog Input.
11. Remove the external Sheath and Waste containers from the SMS.
12. Adjust the **VR2 Sheath Tank Level Offset** to the lowest voltage without going negative.
13. Adjust the **VR1 Waste Tank Level Offset** to the lowest voltage without going negative.
14. Place an empty container on the SMS Sheath load cell and note the analog value
15. Record the **Sheath Tank Level** (0.6v is normal).
16. Place an empty container on the SMS Waste load cell and record the analog value
17. Record the **Waste Tank Level** (0.6v is normal).
18. Place a full sheath container on the SMS sheath load cell and record the analog value
19. Record the **Sheath Tank Level** (8.2v is normal).
20. Place a full waste container on the SMS waste load cell and record the analog value
21. Record the **Waste Tank Level** (8.2v is normal).
22. Place an empty Cubitainer on the Sheath load cell and record the analog value
23. Record the **Sheath Tank Level** (0.6v is normal)
24. Place a full Cubitainer on the Sheath load cell and record the analog value
25. Record the **Sheath Tank Level** (9.10 v is normal)
26. Click on the Start Button, Click on the RUN button and Enter regedit in the dialog box.
27. Click on + HKEY_LOCAL_MACHINE\+SOFTWARE\+Dako\+ Summit.
28. Click on the version of software that is being used.
29. Click on + Cytometer and then CyAn.
30. Click on the Sheath Tank Type in the pane on the right side.
31. Select the style of sheath tank (0 = cubitainer. 1= plastic container). Boxes of water (Type 1A) are called cubitainers. Make sure the Decimal button is selected.



32. Click on the Waste Tank Type in the pane on the right side.
33. Select the style of waste container (0=cubitainer, 1= plastic container).
34. Click on the Sheath Tank Type 1 = reusable plastic, voltage = tank empty.
35. Enter analog voltage from Step 10 (Example: 0.6v = 6 decimal).
36. Click on the Sheath Tank Type 1= reusable plastic, indicating tank full.
37. Enter the analog voltage from Step 14 (Example: 8.2v = 82 decimal).
38. Click OK.
39. Click on Waste Tank Type 1, voltage indicating tank empty.
40. Enter the analog voltage from Step 12 (Example: 0.6v = 6 decimal).
41. Click OK.
42. Click on the Waste Tank Type 1, voltage indicating tank full.
43. Enter analog voltage from step 16 (Example: 8.2v = 82 decimal).
44. Click on Sheath Tank Type 0 (cubitainer), tank empty.
45. Enter the voltage from Step 18. (Example: 0.6 is 6 decimal)
46. Click on Sheath Tank Type 0 (cubitainer), tank full.
47. Enter the voltage from Step 20. (Example: 9.7v is 97 decimal)
48. Click OK.
49. Close regedit.

Acceptance Criteria:

1. Step 2: Able to set TP 49 voltage to +12 +/- 0.1 VDC
2. Step 4: Able to set TP 50 voltage to -12 +/- 0.1 VDC
3. Step 7: Able to adjust the Sheath Tank Level
4. Step 8: Able to adjust Waste Tank Level
5. Step 9: Sheath Tank Level responds to weight change
6. Step 11: Waste Tank Level responds to weight change

Appendix A

Installation Requirements

CyAn ADP Installation Requirements

IMPORTANT
Your Dako representative is responsible for uncrating, installing, and initial set up of the CyAn ADP.

General Laboratory Information

Heating and air conditioning vents or fans are not recommended directly above the CyAn ADP because of the resulting temperature fluctuation, vibration, and possible dust.

Table A.1: CyAn ADP Installation Requirements

General Requirements	
Power Requirements	100V – 240V, 600VA(W), 50/60 Hz
Lab Bench	Stable bench/table top to support the CyAn ADP, one or two monitors, keyboard, and a mouse pad
Service Access	45.7 cm (18 in) minimum around instrument components
Phone	Location near CyAn ADP for contacting technical support
Internet Access	Internet Service Provider or LAN connection for downloading software updates
Dimensions (not including Auxiliary Components)	Height front cover closed - 39.1 cm (15.4 in) front cover open – 72.1 cm (28.4 in) Width - 33.3 cm (13.1 in) Depth - 49.8 cm (19.6 in)

	with clearance for cables – 62.5 cm (24.6 in) Weight - 36.3 kg (80 lbs)
Auxiliary Components	
Sheath Management System (with casters): Houses sheath container, waste container, cleaner fluid container, air compressor, and vacuum	Height – 61.3 cm (24.2 in) Width - 73.3 cm (28.9 in) Depth – 61.9 cm (24.4 in) Weight – 22.6 kg (50 lbs)
Summit Workstation	Height – 42.9 cm (16.9 in) Width – 19.1 cm (7.5 in) Depth – 45.7 cm (18.0 in) Weight – 10.5 kg (23 lbs)
Uninterruptible Power Supply	Height – 20.3 cm (7.9 in) Width – 14.7 cm (5.7 in) Depth – 44.5 cm (17.5 in) Weight – 20 kg (44 lbs)
Operating Environment	
Ambient Temperature	15 to 30°C (59 to 86°F) For optimum performance maintain at +/- 2°C
Relative Humidity	20 to 80% RH (non-condensing)

CyAn ADP Installation Requirements (UV Model)

General Laboratory Information

Heating and air conditioning vents or fans are not recommended directly above the CyAn ADP because of the resulting temperature fluctuation, vibration, and possible dust.

Table A.2: CyAn ADP Installation Requirements (UV Model)

General Requirements	
Power Requirements	100V – 240V, 600VA(W), 50/60 Hz
Lab Bench	Stable bench/table top to support the CyAn ADP, one or two monitors, keyboard, and a mouse pad
Service Access	45.7 cm (18 in) minimum around instrument components
Phone	Location near CyAn ADP for contacting technical support
Internet Access	Internet Service Provider or LAN connection for downloading software updates
Dimensions (not including Auxiliary Components)	Height front cover closed - 39.1 cm (15.4 in) front cover open – 72.1 cm (47.0 in) Width – 119.4 cm (13.1 in) Depth – 59.2 cm (23.3 in) with clearance for cables – 132.1 cm (52.0 in) Weight – 86.5 kg (190 lbs)
Auxiliary Components	

General Requirements	
Sheath Management System (with casters): Houses sheath container, waste container, cleaner fluid container, air compressor, and vacuum	Height – 61.3 cm (24.2 in) Width - 73.3 cm (28.9 in) Depth – 61.9 cm (24.4 in) Weight – 52 kg (115 lbs)
Summit Workstation	Height – 42.9 cm (16.9 in) Width – 19.1 cm (7.5 in) Depth – 45.7 cm (18.0 in) Weight – 10.5 kg (23 lbs)
Uninterruptible Power Supply	Height – 20.3 cm (7.9 in) Width – 14.7 cm (5.7 in) Depth – 44.5 cm (17.5 in) Weight – 20 kg (44 lbs)
Operating Environment	
Ambient Temperature	15 to 30°C (59 to 86°F) For optimum performance maintain at +/- 2°C
Relative Humidity	20 to 80% RH (non-condensing)

CyAn ADP (UV Model) Coherent Enterprise Laser Power Requirements

A dedicated 208-240V single phase 50 amp circuit is required. The laser is hardwired into a junction box with fused disconnect. The junction box must be within 6 feet of the CyAn ADP. An electrician must be available to connect and ensure adequate power for the Enterprise laser on the first day of CyAn ADP installation.

CyAn ADP (UV Model) Coherent Enterprise Laser Ambient Air and Cooling Water Specifications

The Enterprise laser is a water-cooled laser that requires very stable temperatures of the ambient air and the cooling water.

CyAn ADP (UV Model) Heat Exchanger

The CyAn ADP UV model comes with a Coherent Laser Pure 5 (LP5) water-to-air heat exchanger. The LP5 generates 17,000 BTU/hour that needs to be dissipated as efficiently as possible. Laser stability is dependent on the constant temperature of the water circulating through the laser cavity.

Because the water is cooled through a water-to-air heat exchanger, the ambient temperature and humidity must be constant. Heating and air conditioning vents or fans are not recommended directly above the CyAn ADP UV model because of the resulting temperature fluctuation.
Table 2.3 lists some helpful guidelines for optimum laser performance.

Table A.3: Coherent LP5 Optimum Conditions

Item	Description
Ambient Temperature Specification	+/- 2°C
Heat Dissipation	One meter (3 ft) of clearance on all sides for proper air flow. If the laboratory containing the CyAn ADP is too small or the air conditioning not adequate to maintain +/- 2°C, the LP5 comes with 25 ft of hose that allows for the heat exchanger to be placed in an adjacent room. The adjacent room must have thermal stability. The water hoses to the heat exchanger cannot be greater than 50 ft long.
Other Options	The cooling hoses can be hooked up to a variety of heat exchange systems or “chillers.” The water system cooling the laser should be 10-35°C with stability of +/- 1%.

Appendix B

Technical and Instrument Specifications

The technical and instrument specifications for the CyAn ADP are summarized in the following tables.

Table B.1: CyAn ADP UV Model Technical Specifications

Performance	
Acquisition rate	Up to 50,000 events/sec
Excitation Optics	
Optical parameters	2 scatter and 9 fluorescence
Beam geometry	Elliptical
Number of excitation lines	3
Laser nominal operating output (See Coherent 621 Operator's Manual for more information.)	488 nm (150mW argon), UV (50mW argon), 635 nm (25mW semiconductor)
Laser maximum output value (Accessible in the interior of instrument)	488nm – 2W 351nm – 60mW 635nm – 27.5mW
Detectors and Filters (User-selected)	
488 nm Excitation	FL1-530/40 nm, FL2-575/25 nm, FL3-613/20 nm, FL4-680/30 nm, FL5-750 nm
UV Excitation	FL6-400/40 nm, FL7-450/50 nm
635 nm Excitation	FL8-665/20 nm, FL9-750 LP
Signal Processing	

Compensation	9 x 9 full matrix
Signal resolution	65536 (Summit displays up to 4096 channels on all parameters)
Data acquisition channels	11
Fluidics	
Fluidics control system	Software, hardware, and smart-sensor controls provide ease of use with RUN and PAUSE modes
Sample flow rate	Up to 300 µL/min
Sheath fluid	Maximum 1.03 bar (15 psi), nominal 0.41 bar (6 psi)
Quartz cuvette	UV-grade fused silica with 250 µm square-sectioned internal channel
Summit Workstation	
Platform	Windows® XP
Processor	1.7 GHz or faster
Memory	1 GB RAM (minimum)
Storage space	2 60 GB hard drives (minimum), CDRW
Monitor	17-inch LCD flat screen (dual monitor also available)
Network	High-speed Ethernet 10/100 MB/sec

Table B.2: CyAn ADP Technical Specifications

Performance	
Acquisition rate	Up to 50,000 events/sec
Excitation Optics	
Optical parameters	2 scatter and 9 fluorescence
Beam geometry	Elliptical
Number of excitation lines	3
Laser options nominal operating output (See Coherent OPSL Operator's Manual for more information.)	488 nm (20 mW Semiconductor), 635 nm (25 mW semiconductor), 405 nm (25 mW semiconductor)
Laser maximum output value (Accessible in the interior of instrument)	488nm - 20mW 635nm – 27.5mW 405 nm – 25mW
Detectors and Filters (User-selected)	
488 nm Excitation	FL1-530/40 nm, FL2-575/25 nm, FL3-613/20 nm, FL4-680/30 nm, FL5-750 nm
405 nm Excitation	FL6-450/50 nm, FL7-530/40
635 nm Excitation	FL8-665/20 nm, FL9-750 LP
Signal Processing	
Compensation	9 x 9 full matrix
Signal resolution	65536 (Summit displays up to 4096 channels on all parameters)
Data acquisition channels	11

Fluidics	
Fluidics control system	Software, hardware, and smart-sensor controls provide ease of use with RUN and PAUSE modes
Sample flow rate	Up to 300 µL/min
Sheath fluid	Maximum 1.03 bar (15 psi), nominal 0.41 bar (6 psi)
Quartz cuvette	UV-grade fused silica with 250 µm square-sectioned internal channel
Summit Workstation	
Platform	Windows® XP or NT
Processor	1.7 GHz or faster
Memory	1 GB RAM (minimum)
Storage space	2 60 GB hard drives (minimum), CDRW
Monitor	17-inch LCD flat screen (dual monitor also available)
Network	High-speed Ethernet 10/100 MB/sec

Table B.3: CyAn ADP Instrument Specifications

CyAn ADP Enclosure	
Type	Molded RIM polyurethane structural foam
Installation	Indoor only
UV Model Dimensions (not including Utility Cart, Heat Exchanger, or Summit Workstation)	Height 39.1cm (15.4 in) front cover closed 72.1cm (28.4 in) front cover open Width 119.4cm (47.0 in) 132.1cm (52.0 in) including clearance for cables Depth - 59.2cm (23.3 in) Weight - 86.5 kg (190 lbs)
Dimensions (not including Utility Cart or Summit Workstation)	Height 39.1cm (15.4 in) front cover closed 72.1cm (28.4 in) front cover open Width - 33.3cm (13.1 in) Depth 49.8cm (19.6 in) 62.5cm (24.6 in) including clearance for cables. Weight - 36.3 kg (80 lbs)
Auxiliary Components	
Laser Power Supply (UV Model)	Height – 19 cm (7.5 in) Width – 43 cm (17 in) Depth – 61 cm (24 in) Weight - 31 kg (68 lbs)
Sheath Management System (with casters): Houses sheath container, waste container, cleaner fluid	Height – 61.3 cm (24.2 in) Width - 73.3 cm (28.9 in) Depth – 61.9 cm (24.4 in)

container, air compressor, laser power supply, and vacuum	Weight – 52 kg (115 lbs)
Uninterruptible Power Supply	Height – 20.3 cm (7.9 in) Width – 14.7 cm (5.7 in) Depth – 44.5 cm (17.5 in) Weight – 20 kg (44 lbs)
Summit Workstation	Height - 42.9cm (16.9 in) Width - 19.1cm (7.5 in) Depth - 45.7cm (18.0 in) Weight - 10.5 kg (23 lbs)
External Transformer	Height – 19.1cm (7.5 in) Width - 31.2 cm (12.3 in) Depth - 19.1 cm (7.5 in) Weight - 11.4 kg (25 lbs)
Safety	
Interlock	The front cover is protected with dual magnetic positive-break (➲) type interlock switch. Operates dual spring solenoid shutter actuators on all laser paths.
Laser product class	CLASS 1 LASER PRODUCT IEC/EN 60825 -1/A2:2001
Laser light leakage	Conforms to 21CFR1040.10 and 21CFR1040.11
Operating Environment	
Ambient temperature	15 to 30°C (59 to 86°F) For optimum performance maintain at +/- 2°C
Relative humidity	20 to 80% RH (non-condensing)

CyAn ADP System Utility Requirements & Fusing	
Power, UV Model (not including Enterprise II 621 Laser)	115 VAC +/- 10%, 60 Hz, single phase, 1.5A
Power, standard ADP	115 VAC +/- 10%, 60 Hz, single phase, 1.75A
Fuse	Type – 5x20mm, low breaking, high capacity, IEC. 115 VAC operation – 6.3A
Power, Sheath Management System	100-240 VAC , 50/60 Hz, single phase, 2A
Fuse, Sheath Management System	Type – 5x20mm, low breaking, high capacity, UL. 115 VAC operation – 3A
Power, Summit Workstation (not fused)	115 VAC +/- 10%, 60 Hz, single phase, 1A
Power, Summit Workstation Monitor (not fused)	115 VAC +/- 10%, 60 Hz, single phase, 2A
Power, External Transformer	Input: 230 VAC +/- 10%, 50/60 Hz, 5A Output: 15 VAC +/- 10%, 60 Hz, 8A
Fuse, External Transformer power	Type – 5x20mm, low breaking, high capacity, IEC, 115 VAC operation – 6.3A
CyAn ADP UV Model Laser System Utility Requirements	
Power, Coherent Enterprise II 621 Laser	208 – 240VAC, single phase, 50A
Cooling method	Water
Cooling load	4.5 kW (15,000 Btu/hr)
Cooling water pressure	138 – 414 kPa (20 – 60psi)
Cooling water inlet temperature	10 – 60 °C (50 – 140 °F)

Cooling water flow rate	8 – 11.6 l/min (2 – 3 gpm)
CyAn ADP UV Model Heat Exchanger Option	
Coherent LP5 Heat Exchanger	Height - 54.0cm (21.0 in) Width - 69.0cm (27.0 in) Depth - 44.0cm (17.0 in) Weight - 50 kg (110 lbs)
Power, Coherent LP5 Heat Exchanger	110/220 VAC +/- 10% 50/60 Hz, 10A
Fuse, Coherent LP5 Heat Exchanger power	Type – 5x25mm, fast acting, high capacity, IEC. 115 VAC operation – 5A 230 VAC operation – 2.5A
Cooling Method	Water-to-Air Heat Exchange
Heat Output	5 kW (17,000 Btu/hr)
Cooling water flow rate	8 – 9.5 l/min (2 – 2.5 gpm)
Maximum laser water return temperature	68 °C (154.4 °F)
Maximum ambient air temperature (heat exchanger only)	40 °C (100 °F)

Appendix C

Glossary

Cytometry	The measurement of cellular characteristics.
Flow Cytometry	Measurements of cellular characteristics made as cells or other particles in a fluid suspension pass one by one through a measurement apparatus, or cytometer.
Histogram	A histogram is plotted on two axes and is used to represent bins or channels of aggregated data. Typically these bins contain data within a specified range of values. The histogram itself gives information on the number of data values within a particular bin.
Light Scatter	Redirection of light due to its interaction with matter.
Fluorescent Light	Light emitted from a molecule due to excitation from a lower wavelength light source.
Fluorescent label	A probe with a fluorescent tag designed specifically to bind to your cell or cell component of interest at a given site.
Excitation Wavelength	This is the wavelength of light that a fluorescent label or cell will absorb; thus sometimes referred to as “absorption wavelength”.
Emission Wavelength	This is the wavelength of light that a fluorescent label or cell will emit. It is always longer (more toward the red end of the spectrum) than the excitation wavelength for a particular dye or stain.
Sheath Fluid	This is a basic saline solution used to carry the sample through the nozzle in a laminar way. Most are similar to PBS. CytoFlo™ is Dako’s preservative-free phosphate buffered saline.
Photomultiplier Tube (PMT)	This is the component of a flow cytometer that looks for light flashes and sends them to the electronics for processing.
Photodiode	This is usually used instead of a PMT for Forward Scatter. It is somewhat less sensitive but is sufficient for Forward Scatter detection.
Optical Filter	This is a piece of dyed/coated glass that allows only certain wavelengths of light to pass through while

	rejecting or deflecting all others. They are a very important part of a cytometer and are used in front of the detectors. Neutral density filters attenuate intensity without affecting wavelength.
Oscilloscope	An oscilloscope is a instrument that displays data as it occurs either in a dot plot or a pulse form.
FCS file	FCS stands for “Flow Cytometry Standard”. It is the file format used throughout the flow world so different software can read the same files.
Gate	A gate is a region (ellipse, rectangle, polygon, etc.) that is placed around a population of interest to isolate and gain statistics from that data. When a histogram is “gated” it means that only the data within the region is displayed in the other histograms. This helps to eliminate background noise or contamination.
CyAn ADP	“Cyto Analyzer Advanced Digital Processing”
Flow Cell	The entire assembly containing cuvette, upper body, and lower body.
Cuvette	The quartz chamber through which sheath fluid and cells flow through the laser beam
Summit	Dako’s powerful acquisition and analysis software package. This is where you see your data in its final form.
Layout	A template specific to particular types of samples run in Summit. Any number can be made in the Summit database and each consists of all the histograms, gates, regions, compensation and sort decisions for a given procedure/test/investigator, etc.
Layout Navigator	The Layout Navigator is a powerful window with a simple function: storage and retrieval of the Page (screen) Layouts. In addition you’ll find gates, statistics and other settings available here.
Data Navigator	The Data Navigator lets you easily navigate and interact with your database. Acquired data will be stored here for analysis as well as imported FCS files. This is also the place where LIN/LOG information and parameter names are stored.
Database	Summit’s database is the collection of information that the scientist or operator needs to see from session to session, or does not want to have to redefine every day.

This includes instrument settings, layouts, sample information, etc. The Layout and Data Navigators will help to organize the information within the Database.