ETOGN.001VPC PATENT

HIGH SPEED SAMPLE SUPPLY DEVICE

CROSS-REFERENCE TO RELATED APLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/781,103, entitled "High Speed Sample Supply Device" and filed on March 10, 2006, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] This invention relates to a sample supply device for supplying an inspection instrument, such as a flow cytometer, with a plurality of samples at high speed.

Description of the Related Art

[0003] Traditionally, sample supply to the inspection cuvette of a flow cytometer has been accomplished by pressurizing a tube containing a sample to be analyzed, causing the sample to flow into the sample supply line. Automation of this traditional supply method is difficult due to mechanical issues with automatic positioning and sealing of sample containers. Such a supply method is also prone to creating aerosols of biohazardous materials.

[0004] Another method of sample supply, which is sometimes implemented in flow cytometers, is aspiration of sample into the flow cytometer syringe pump and then expulsion of the sample into flow cytometer cuvette. One drawback of this method is that pulsations created in the sample flow can greatly amplify noise on the measured signal. Additionally, the amount of time required for washing between samples is great and slows the process for analyzing samples. The washing process cannot begin until sample has been fully dispensed into the flow cytometer cuvette, resulting in reduced throughput.

[0005] Another method of sample supply is described in U.S. Patent No. 5,182,617, which is herein incorporated by reference in its entirety. The '617 Patent discloses that higher throughput may be achieved by creating two identical branches that can perform simultaneous sample introduction and washing. When used, analyzed samples stick to the channels of the system, including the interface channels, causing major carryover issues in subsequent sample analysis. The prior art provides no provision for thorough washing of an interface channel between the sample supply device and the

flow cytometer. Furthermore, the prior art fails to teach any provision for rapidly washing the system with a cleaning liquid.

SUMMARY OF THE INVENTION

[0006] A method for multiplexed sample analysis is provided comprising the steps of (a) robotically obtaining a first liquid sample from the first of a plurality of sample sources; (b) delivering the first liquid sample through a first line to an instrument for analysis of the sample (c) robotically obtaining a second liquid sample from the second of a plurality of sample sources; (d) delivering the second liquid sample through a second line to an instrument for analysis; (e) cleaning the second line while the first line is delivering sample to the instrument; and (f) cleaning the first line while the second line is delivering sample to the instrument. In one embodiment, the method further comprises step (g) repeating steps (a)-(f) to obtain and deliver multiple samples to the instrument from all of the plurality of sample sources. Preferably, the instrument is a flow cytometer. In another embodiment, the instrument is selected from the group consisting of a spectrofluorometer, a fluorometer, an absorbance meter, and a microscope.

[0007] In an embodiment, the first and second liquid samples that are analyzed by the inspection instrument may be independently selected from the group consisting of chemical compounds, antibodies, beads, live cells, or fixed cells. Any sample typically analyzed by a flow cytometer, HPLC, spectrofluorometer, fluorometer, absorbance meter, microscope., or other high-throughput-instrument that receives liquid samples may be used.

[0008] In another embodiment, the method further comprises the steps of delivering a first reagent to the first liquid sample before the sample is delivered to the instrument and delivering a second reagent to the second liquid sample before the sample is delivered to the instrument. The first and second reagents may be independently selected from the group consisting of chemical compounds, antibodies, beads, live cells, or fixed cells. In still another embodiment, multiple reagents may be added to the sample before the sample is injected into the instrument.

[0009] In another embodiment, a control valve alternates delivery of cleaning fluid with the delivery of liquid sample in each of the first and second lines. The control valve may be connected to additional lines. Such lines may further provide the delivery

of air pressure or system liquid pressure. Additionally, such lines may further provide a waste channel.

[0010] An apparatus for delivering samples to an instrument for analysis is provided comprising a first and a second sample delivery line, wherein the first sample delivery line comprises a first sample loading and injection branch and the second sample delivery line comprises a second sample loading and injection branch; a fluid flow management mechanism for alternately connecting the first and second sample delivery lines to the instrument; at least one cleaning fluid delivery line to supply cleaning fluid to the flow management mechanism, the first sample delivery line, and the second sample delivery line; at least one sample input channel; at least one control valve for alternately delivering cleaning fluid to the first and second sample delivery lines, so that sample and cleaning fluid alternately flow through the first and second sample delivery lines. In one embodiment, the at least one cleaning fluid delivery line supplies cleaning fluid to the entire system, including the inspection instrument.

[0011] In another embodiment, the fluid flow management mechanism comprises a position switching valve. The position switching valve may comprise a four-way two-position switching valve. The two-position switching valve may comprise two modes. In one embodiment, the first mode of the position switching valve fluidicly couples the first sample delivery line to the instrument and fluidicly couples the second sample delivery line to the sample input channel. In another embodiment, the second mode of the position switching valve fluidicly couples the first sample delivery line to the sample input channel and fluidicly couples the second sample delivery line to the instrument.

[0012] In one embodiment, the sample loading and injection branches of the first and second sample delivery lines comprise symmetrical sample loading and injection branches. In another embodiment, the first sample delivery line comprises a first sample holding loop and the second sample delivery line comprises a second sample holding loop. In another embodiment, a first control valve is located within the first sample delivery line and a second control valve is located within the second sample delivery line. In some embodiments, the first and second control valves each comprise four fluidic channels capable of coupling the first and second sample holding loops to one of the four fluidic channels. These additional fluidic channels may be connected to output ports of

pump flow control valves, at least one cleaning fluid delivery line, waste disposal, or a controlled air pressure source.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 is a diagram showing a configuration of one embodiment of the present invention.

[0014] FIGS. 2A and 2B show a top level diagram showing an embodiment of one operation of the control unit.

[0015] FIG. 3 is a diagram showing an embodiment of one sequence of control unit commands for washing both branches of the system.

[0016] FIG. 4 is a diagram showing an embodiment of one sequence of control unit commands for adding a reagent to sample containers on both branches of the system.

[0017] FIG. 5 is a diagram showing an embodiment of one sequence of control unit commands for mixing and loading samples on both branches of the system; and

[0018] FIGS. 6A and 6B show a diagram showing an embodiment of one sequence of control unit commands for injecting sample and washing interface line on both branches of the system.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0019] The term "pressurizing means" as used herein refers to a variety of methods for providing pressure into the channels of the device. Non-limiting examples of pressurizing means include regulated compressed air from a tank, a regulated air compressor, and a syringe pump.

[0020] The term "storage means" as used herein refers to a variety of sample containers from which sample is aspirated into the sample supply channel. Non-limiting examples of storage means include a 96, 384, or 1536 well plate or a rack comprising any number of test tubes.

[0021] The term "channel switching means" as used herein refers to a switching valve that directs fluid in the channels to desired locations in the device of any embodiment disclosed herein.

[0022] FIG. 1 shows one embodiment of the present invention and is intended to be non-limiting in its scope. In FIG. 1, an inspection instrument (in this case a flow cytometer) **1** is in fluid communication with a four-way two position switching valve **3**

via a flexible fluidic interface channel **2**. The interface channel may be constructed of any known tubing material suitable for use with an inspection instrument. Non-limiting examples of tubing material for the interface channel and all other tubing materials involved in the present invention include tubing made from flexible silicon, polyvinyl chlorine (PVC), polyether ether ketone (PEEK), polytetrafluoroethylene (PTFE), and any other useful polymeric materials, such as polyesters, polyolefins, or polyamides. The interface channel **2** may have an internal diameter from roughly about .005" to about 0.040", although other sizes of internal diameter may be appropriate depending on the inspection instrument used. The valve **3** switches sample input channel ("input channel") **4** and interface channel **2** between two symmetrical sample loading and injection branches of the system **5** and **6**. In a first position of the switching valve **3**, branch **5** is fluidicly coupled to input channel **4** and interface channel **2** (and thus, the inspection instrument **1**) is fluidicly coupled to branch **6**. When the valve **3** is switched into a second position (not shown), branch **5** would be fluidicly coupled to interface channel **2** (and thus, the inspection instrument **1**) and input channel **4** would be fluidicly coupled to branch **6**.

[0023] The other end of input channel **4** and is coupled to a probe **7**, which is mechanically coupled to a positioning device **8**. In one embodiment, the input channel comprises the probe and positioning device. The probe **7** provides the input channel **4** with an appropriate sample. Any type of sample that is analyzed with by an inspection instrument may be used in the present invention, including liquid, gaseous, or solid samples. Non-limiting examples of useful samples include chemical compounds and biological compounds. Some non-limiting examples of chemical compounds include chemical reagents, solvents, microspheres, beads, and dyes. Preferably, the chemical compounds comprise compounds that are known and used in an HPLC or a flow cytometer inspection instrument. Some non-limiting examples of biological compounds include blood, urine, antibodies, live cells, dead cells, and microorganisms. The cells may be from humans, animals, insects, bacteria, yeast, or viruses. Preferably, the biological compounds comprise compounds that are known and used in a flow cytometer inspection instrument.

[0024] The positioning device **8** may be any device known to one of ordinary skill in the art. The positioning device **8** may be commanded to move the probe **7**. Non-limiting examples of positioning devices include a Cartesian robotic sampler and a rotary sampler. Another example of a positioning device **8** includes an XYZ positioning device,

such as a Tecan MSP9250 robotic sampler with travel range of 15.4" on X axis, 11.8" on Y axis, and 6.5" on Z axis. The positioning accuracy of this device is 0.004" on all axes. The Z axis of positioning device **8** is capable of mounting probes of diameters up to 0.078" via a set screw. In another embodiment, the robotic sampler comprises separate arms for delivering a variety of reagents to the samples. In another embodiment, the robotic sampler comprises separate arms, for example, one, two, three, or four arms, for delivering a variety of samples to the inspection instrument. In another embodiment, the at least one sample input channel is coupled to a robotic sampler positioning device. In another embodiment, the robotic sampler comprises two arms and the second arm comprises a reagent transfer pump. In one embodiment, the second arm delivers a variety of reagents, for example, first and second reagents. In another embodiment, the reagent transfer pump comprises a syringe pump, a peristaltic pump, or a diaphragm pump.

[0025] Probe **7** may be any known tubing systems useful in the transportation of samples within the system. In one embodiment, probe **7** comprises a stainless steel tubing of about 0.072" outer diameter (OD), and about 0.063" inner diameter (ID), although other tubing dimensions are contemplated. For example, the OD of the probe may range between about 0.050" and 0.100" and ID of the probe may range between about 0.045" and about 0.095". A typical probe tubing length is about 4" but could be longer or shorter depending on the type of sample containers **9** and reagent vials **11** that are used. Preferably, probe **7** is long enough to be able to reach the bottom of sample containers **9** and reagent vials **11** without end of Z axis impacting top of the container. An additional 1" may be added to this minimum probe length to allow for mounting of probe **7** in the end of Z axis of positioning device **8**.

[0026] In one embodiment, probe **7** has a rectangular opening machined into its side, starting about 1" from the end of probe **7** where it is mounted onto the Z axis of positioning device **8** and extending for about 0.5" from that point. The depth of this opening may be about one half of the outer diameter of the probe **7**. Input channel **4** may pass through this opening and out of the tip of probe **7**. The outer diameter of input channel **4** may be sized so that it snugly fits within the inner diameter of the probe **7**. For example, the OD of input channel **4** may be about 0.0625" when the ID of the probe is 0.063" and the input channel **4** may optionally be friction fit into probe **7**. Input channel **4** may be extended from the tip of probe **7** by approximately 0.5" to avoid contact between

the outer wall of probe **7** and the contents of sample containers **9** where the sample containers are relatively flat, for example, a 96-well plate, a 385-well plate, or a 1536-well plates.

[0027] The positioning device **8** can be commanded to move probe **7** among several devices, including sample containers **9**, a wash station **10**, and any of the reagent vials **11** located in a reagent rack **12**. Many different sample containers **9** may be employed in the embodiments described herein. Non-limiting examples of sample containers include tube racks comprising varying amounts of sample tubes and well plates of 96, 384, or 1536 wells.

[0028] In one embodiment, the wash station **10** is coupled to a waste reservoir **13** via a large bore drain channel **14**. In another embodiment, the waste reservoir **13** is physically located below the wash station **10** to allow any liquids expelled into the wash station **10** to flow into the waste reservoir **13** under gravity. The waste station drain channel should have an internal diameter large enough to prevent airlock in the line upon drainage of any waste material. The typical internal diameter of a waste station drain channel **14** is not less than about 3/8 inches.

[0029] In an embodiment, both sample loading and injection branches **5** and **6** of the system are identical in configuration and are each independently controlled. In an embodiment, each branch **5** and **6** consists of a sample holding loop **15** and **16**, respectively, control valves **17** and **18**, respectively, channels **19** through **22** and **23** through **26**, respectively, pump flow control valves **27** and **28**, respectively, and pumps **29** and **30**, respectively. In one embodiment, the sample holding loops **15** and **16** are fluidicly coupled to the switching valve **3**. In an embodiment, the sample holding loops **15** and **16** are constructed of a length of tubing having internal volume greater than the maximum volume of sample that can be loaded and injected by the system. This ensures that the sample holding loops **15** and **16** have sufficient volume to contain any sample that may be injected therein.

[0030] The other end of each holding loop **15** and **16** is fluidicly coupled to control valves **17** and **18** respectively. Typically, holding loops **15** and **16** would have internal volume one and one half times greater than the maximum volume of sample to prevent sample from being aspirated into control valves **17** and **18**. In one embodiment, both control valves **17** and **18** are selection valves comprising four channels capable of

coupling sample holding loops **15** and **16** to one of the four fluidic channels. For embodiments not requiring as many fluidic channels, the selection valves may comprise as few as two channels. In other embodiments, selection valves with more than four channels can be used in order to provide additional washing capabilities of the system.

[0031] Control valves **17** and **18** having multiple channels provide many advantageous features for the invention described herein. In FIG. 1, control valves **17** and **18** selectively connect sample holding loops **15** and **16** to one of four possible channels. Holding loop **15** is connected to channels **19**, **20**, **21**, and **22** and holding loop **16** is connected to channels **23**, **24**, **25**, and **26**.

[0032] In an embodiment, the control valves **17** and **18** comprise channels for pumps. Pump channels **19** and **23** are connected to output ports of pump flow control valves **27** and **28** respectively. Pump flow control valves **27** and **28** are normal three way valves with a common port, a normally open port, and a normally closed port. The common ports of pump flow control valves **27** and **28** are connected to pumps **29** and **30**, which are capable of aspirating and dispensing liquids. In an embodiment, pumps **29** and **30** are each independently a syringe pump. However, other types of pumps may be used. Non-limiting examples include peristaltic pumps and diaphragm pumps. In an embodiment, the input ports of pump flow control valves **27** and **28** are connected to a system liquid reservoir **31**. Any system liquid useful in allowing or aiding performance of the inspection instrument may be used. Non-limiting examples include water, saline, or phosphate-buffered saline.

[0033] In an embodiment, the control valves **17** and **18** comprise channels for holding cleaning fluid to wash and clean the system. Wash channels **20** and **24** are both fluidicly coupled to each other and a wash pump **32** at tee junction **33**. The wash pump **32** is fluidicly coupled to a common port of a three way wash liquid selection valve **34** that comprises a common port, a normally open port, and a normally closed port. The normally open port of valve **34** is connected to system liquid reservoir **31** via channel **35** and the normally closed port of valve **34** is connected to cleaning liquid reservoir **37** via channel **36**. Wash pump **32** in this embodiment is a diaphragm pump capable of rapidly pumping cleaning liquids to control valves **17** and **18**. Other non-limiting examples of suitable pumps that can be used for pumping liquids through the system include peristaltic, syringe, and a pressurized reservoir.

[0034] In an embodiment, the control valves **17** and **18** comprise channels for waste. Waste channels **21** and **25** are both fluidicly coupled to each other and a waste reservoir **13** at a tee junction **38**.

[0035] In an embodiment, the control valves **17** and **18** comprise channels for controlling the pressure in the channels. Pressurized channels **22** and **26** are both fluidicly coupled to each other and a pressurized system liquid reservoir **39** at a tee junction **40**. The pressurized system liquid reservoir **39** is pressurized via a carefully controlled air pressure source **41**. Controlled air pressure sources useful in the present invention include a regulated compressed air tank or a regulated air compressor. Alternatively, the pressure source may come from the inspection instrument **1** if such source is available. However, pressurized channels **22** and **26** are preferred when very precise measurements are to be taken and injecting samples with pumps **29** and **30** would increase the signal to noise ratio.

[0036] One improvement provided by the control valves **17** and **18** is the capability to rapidly wash lines **15** and **16** with a cleaning liquid **37**. A sticky compound is any compound that remains in the lines of the system that has the potential to contaminate future samples. Often, sticky compounds, for example compounds such as sphingosine-1-phosphate (S1P), endothelin-1 (ET-1), and rhodamine, are involved when using inspection instruments, such as flow cytometers. It is therefore essential to clean system lines between sample inspections with a cleaning solution in order to eliminate any carryover that may exist from previous samples. Typical system liquids, such as saline solution, as compared to cleaning liquids, are prone to leave behind sticky compounds in the lines of the system. This causes contamination for hundreds of samples that are analyzed afterward.

[0037] Another feature of the control valves **17** and **18** is that they allow the inspection instrument **1** to back-flush remaining sample in interface line **2** back through the apparatus into waste. As sample travels through interface line **2** and lines **15** and **16**, it becomes longer because sample in the center of the tube travels faster than at the surface. This often leads to a situation where the whole sample is never fully injected into the inspection instrument.

[0038] This may be accomplished by connecting line **2** to one of the lines **15** and **16** and also connecting control valves **17** and **18** to waste channels **21** and **25**. Where

a flow cytometer is used as the inspection instrument, it may pressurize the input line **2**, making the connection to a waste line that is open to atmospheric pressure. This often causes a rapid flow of clean liquid from inspection instrument **1** through interface line **2**. This feature makes it possible to carry out inspection of samples at high speed because it overcomes the problem of waiting for extended periods of time for sample to clear the tubing.

[0039] Another feature of the control valves **17** and **18** is that they allow the capability to load large sample volumes utilizing syringes **29** and **30**. The large sample volumes may then be injected with high precision utilizing pressurized system liquid **39**. This is particularly important as syringe size increases. As syringe size increases, the minimum flow rate that a syringe pump can sustain without fluctuations also increases. As the flow rate increases, the inspection instrument (particularly a flow cytometer) loses precision in measuring the samples. The present invention described herein allows one of ordinary skill in the art to load a large sample, such as 1 mL, 2 mL, or even greater, and then inject it very slowly allowing for the same precision found in manual tube injection, but with the additional advantage of full automation.

[0040] In another embodiment, the pressurized system liquid vessel **39** may be replaced with a high-resolution syringe pump. High-resolution syringe pumps are capable of supplying larger volumes of liquid at very low flow rate. Inserting a high-resolution syringe pump may be accomplished by replacement of the pressurized system liquid vessel **39**.

[0041] The invention described herein provides the capability to deliver one or more reagents to sample containers before they are injected into the system by utilizing a separate auto-sampler arm comprising its own syringe for aspirating or dispensing. Such a feature would prevent carryover from sample containers back to the reagent if the auto-sampler arm carrying a reagent dispenses that reagent into the wells above the liquid surface of the sample. In an embodiment, the auto-sampler comprises multiple arms, such as an auto-sampler comprising two, three, or four arms. In another embodiment, the auto-sampler comprises two arms. Where cells are utilized as the reagent and a single same auto-sampler arm is used to take cells to the sample, mix and inject, and then go back to cells, it is possible that carryover of unwashed parts of the sample back to the cells may occur. One example where multiple reagents may be added to the sample before injection in the instrument comprises having agonist compounds on a plate well and adding cells to

the well before they are injected into the inspection instrument. Other combinations of reagents and samples, as described herein, may be substituted for agonists and cells. Another example comprises placing an allosteric modulator or other antagonist compounds in a plate well, adding cells from a reagent vial, and then finally adding another agonist compound into the well. The mixture may then be injected into the flow cytometer. Each sample may have different types and varying numbers of reagents added thereto. In one embodiment, delivery of first and second reagents, and optionally additional reagents is performed with a separate auto-sampler arms.

[0042] In some embodiments, the channels comprising the system are made with polymer tubing having internal diameter of about 0.02 inches. In another embodiment, a PFA (PerFluoroAlkoxy) material is used to minimize any issues associated with gas permeability. Channels connected to the system liquid reservoir **31** are typically made with tubing having larger internal diameter such as about 1/16 inches or about 0.094 inches. In another embodiment, the holding loops **15** and **16** are made out of larger internal diameter tubing if switching valve **3** and control valves **17** and **18** are in close physical proximity in order to reduce the required length of tubing.

[0043] In an embodiment, a control unit **42** is electrically connected to switching valve **3**, positioning device **8**, wash pump **32**, wash liquid selection valve **34**, control valves **17** and **18**, pump flow control valves **27** and **28**, and pumps **29** and **30**. The control unit **42** is implemented as a computer capable of independent control of all attached devices. Control unit **42** may also set the air supply pressure **41** via an adjustable regulator. The air supply pressure can also be controlled via a manual pressure regulator. In an embodiment, the air supply pressure **41** is set to a value slightly higher than backpressure generated by inspection instrument **1**. For example, the air supply pressure may be set to between about 0.5 and about 2.0 psi over the backpressure.

[0044] The operation of the foregoing apparatus having the above-described configuration will now be explained in some non-limiting embodiments. The control of the operation is preferably performed according to commands from control unit **42**.

[0045] Initially, an operation is performed to fill all system channels (except for waste channels **21** and **25**) with system liquid. Positioning device **8** is commanded to move the probe **7** into the wash station **10**. The pump flow control valve **27** is switched to communicate system liquid from reservoir **31** to pump **29**. Thereafter, pump **29** is filled

with system liquid by command from control unit **42**. The pump flow control valve **27** is then switched to communicate system liquid in pump **29** to pump channel **19**. The valve **3** is switched into position to communicate liquid between input channel **4** and holding loop **15**.

[0046] Next, control valve **17** is switched to communicate liquid between holding loop **15** and pump channel **19**. The pump **29** is commanded to completely expel system liquid into pump channel **19** and holding loop **15** effectively purging air and filling both with the system liquid. Valve **17** is then switched to communicate liquid between holding loop **15** and wash channel **20**, which connects to the wash pump **32**. The wash liquid selection valve **34** is switched to fluidicly couple the wash pump **32** and cleaning liquid reservoir **37** via cleaning liquid channel **36**. Wash pump **32** is then turned on to fill cleaning liquid channel **36** with a cleaning liquid. Any cleaning liquid capable of removing sample residue and providing a non-contaminated surface for the channels may be selected. Some non-limiting examples of cleaning liquids include solvents such as ethanol, dimethyl sulfoxide (DMSO), or a detergent. Wash liquid selection valve **34** is then switched to fluidicly couple wash pump **32** and system liquid reservoir **31** via channel **35**. The system liquid fills wash pump **32**, wash channel **20**, control valve **17**, holding loop **15**, switching valve **3**, input channel **4** and probe **7** with system liquid.

[0047] The wash pump **32** is then turned off after a fixed delay sufficient to fill components mentioned above with the system liquid. If pressurized system liquid reservoir **39** is connected to control valve **17**, then valve **17** is switched to communicate the pressurized system liquid from reservoir **39** to holding loop **15** via pressurized channel **22**. After a delay to fill pressurized channel **22** with system liquid, control valve **17** is switched to communicate liquid in holding loop **15** to the waste channel **21**.

[0048] In order to fill the second branch of the system with system liquid, valve **3** is switched into position to communicate liquid between input channel **4** and holding loop **16**. The sequence of events described above for the first branch of the system is then repeated for the equivalent parts of the second branch of the system. The resulting state of the system is with probe **7** in wash station **10** and all channels of the system (except waste channels **21** and **25**) filled with system liquid. In another embodiment, the process of filling lines with system liquids may further be practiced with additional branches.

[0049] FIGS. 2A and 2B show another embodiment of the present invention and are intended to be non-limiting in its scope. FIGS. 2A and 2B show a sequence of steps to evaluate one or more samples from sample containers **9**. In step **43**, which is the starting point of FIG. 2A, a wash cycle **68** (further described in FIG. 3) is executed on branch **5**. Wash cycle **68** ensures that branch **5** has been washed and is filled with system liquid. In some embodiments, it may be beneficial to add a reagent to sample containers **9** before the sample is analyzed. Examples of non-limiting reagents include agonists, antagonists, modulators, dyes, stains, cells, and beads. Where a user selects to add a reagent to the sample in step **44**, a reagent addition cycle **94** is executed on branch **5** in step **45**. In step **46**, the first sample is loaded into holding loop **15** by executing mix and load next sample cycle **110** (further described in FIG. 5) on branch **5**. In step **47**, switching valve **3** is switched to fluidicly couple holding loop **15** and interface channel **2**. Two concurrent and independent processes are then executed. The first process comprises step **48** and the second process comprises steps **49** through **53**.

[0050] In the first process, a sample is injected into the inspection instrument **1** and the interface channel **2** is washed in step **48** by running inject sample and wash interface line cycle **128** (further described in FIG. 6A) on branch **5**. The second process first determines if there are any more samples left to be processed in step **49**. If all of the samples have been processed, the second process terminates by going to step **54**. Otherwise branch **6** is washed in step **50** by executing a wash cycle **81** (further described in FIG. 3). If the user selected to add a reagent to the sample in step **51**, a reagent addition cycle **102** (further described in FIG. 4) is executed on branch **6** in step **52**. In step **53**, the next sample is loaded into holding loop **16** by executing mix and load next sample cycle **119** (further described in FIG. 5) on branch **6**. Upon completion of both processes in step **54**, a check is made in step **55**, shown in FIG. 2B, to determine if all samples have been processed. If no more samples remain, then the execution continues at step **65**.

[0051] If there are still samples to be processed, switching valve **3** is switched to fluidicly couple holding loop **16** and interface channel 2 in step **56**. Two concurrent and independent processes are then executed. The first process comprises step **57** and the second process comprises steps **58** through **62**.

[0052] The first process injects sample into inspection instrument **1** and washes interface channel **2** in step **57** by running inject sample and wash interface line

cycle **137** (further described in FIG. 6B) on branch **6**. The second process first determines if there are any more samples left to be processed in step **58**. If all samples have been processed, then the second process terminates by going to step **63**. Otherwise branch **5** is washed in step **59** by executing a wash cycle **68**. If the user selected to add a reagent to the sample in step **60**, a reagent addition cycle **94** is executed on branch **5** in step **61**. In step **62**, the next sample is loaded into holding loop **15** by executing mix and load next sample cycle **110** (further described in FIG. 5) on branch **5**. Upon completion of both processes in step **63** a check is made in step **64** to determine if all samples have been processed. If no more samples remain the execution continues at step **65**, otherwise execution continues at step **47** described above.

[0053] In step **65**, branch **5** is washed by executing wash cycle **68**. Then, branch **6** is washed by executing wash cycle **81** in step **66**. Sample processing completes in step **67** with the system in a ready state to start processing another set of samples upon replacement of sample containers **9**.

[0054] FIG. 3 shows another embodiment of the present invention and is intended to be non-limiting in its scope. FIG. 3 shows a sequence of steps wherein both branches of the system described herein are washed. The initial wash cycle **68** begins by moving probe **7** to wash station **10** in step **69**. In step **70**, control valve **17** is switched to connect wash channel **20** to holding loop **15**. In some embodiments, samples to be introduced into the inspection instrument **1** may be hydrophobic and prone to sticking to the channel walls. It is often necessary to wash the channels with a cleaning liquid to reduce chances of cross contamination from one sample to the next. Any cleaning liquid capable of removing sample residue and providing a non-contaminated surface for the channels may be selected. Some non-limiting examples of cleaning liquids include solvents such as ethanol, dimethyl sulfoxide (DMSO), or a detergent.

[0055] In step **71**, the wash liquid selection valve **34** is switched to connect cleaning liquid channel **36** and wash pump **32**. The wash pump **32** is then turned on in step **72** to pump cleaning fluids into the control valves. A user specified delay of X1 seconds is introduced at step **73** to allow cleaning liquid to be pumped all the way through valve **17**, holding loop **15**, switching valve **3**, input channel **4** and probe **7** into wash station **10**. The actual amount of time needed may vary depending on the system being used and the length of the channels. One of ordinary skill in the art, guided by the

disclosure provided herein, can determine how much time will be required for the cleaning liquids to be pumped throughout the system.

[0056] With the wash pump **32** still running, the wash liquid selection valve **34** is switched to connect channel **35** to the wash pump **32**, as shown in step **74**. This action establishes a flow of system liquid from system liquid reservoir **31** through the wash pump **32**. A user specified delay of Y1 seconds is introduced at step **75** to allow system liquid to be pumped all the way through valve **17**, holding loop **15**, switching valve **3**, input channel **4** and probe **7** into wash station **10**, thus washing out all remaining cleaning liquid. The actual amount of time needed may vary depending on the system being used and the length of the channels. One of ordinary skill in the art, guided by the disclosure provided herein, can determine how much time will be required to wash out the cleaning liquids.

[0057] Wash pump **32** is turned off in step **76** and control valve **17** is switched to connect pump channel **19** and holding loop **15** in step **77**. In step **78**, pump flow control valve **27** is switched to connect pump channel **19** and pump **29**. All fluid is then expelled out of pump **29** in step **79**. Step **80** shows the completion of wash cycle **68**.

[0058] The wash cycle **81** for branch **6** of the system is the same as wash cycle **68** for branch **5** with all elements of branch **5** replaced with corresponding elements of branch **6**. The wash cycle **81** is illustrated in steps **82** through **93**. The initial wash cycle **81** begins by moving probe **7** to wash station **10** in step **82**. In step **83**, control valve **18** is switched to connect wash channel **24** to holding loop **16**. As previously stated in regards to wash cycle **68**, some embodiments involve samples introduced into the inspection instrument **1** that are hydrophobic and prone to sticking to the channel walls. Similar cleaning liquids may be used in regards to wash cycle **81** that are useful in regards to wash cycle **68**.

[0059] In step **84**, the wash liquid selection valve **34** is switched to connect cleaning liquid channel **36** and wash pump **32**. The wash pump **32** is then turned on in step **85** to pump cleaning fluids into the control valves. A user specified delay of X2 seconds is introduced at step **86** to allow cleaning liquid to be pumped all the way through valve **18**, holding loop **16**, switching valve **3**, input channel **4** and probe **7** into wash station **10**. The amount of X2 seconds used in step **86** may be the same or different from the amount of time used in step **73** for wash cycle **68** on branch **5**. One of ordinary skill

in the art, guided by the disclosure provided herein, can determine how much time will be required for the cleaning liquids to be pumped through the system.

[0060] With the wash pump **32** still running, the wash liquid selection valve **34** is switched to connect channel **35** to the wash pump **32**, as shown in step **87**. This action establishes a flow of system liquid from system liquid reservoir **31** through the wash pump **32**. A user specified delay of Y2 seconds is introduced at step **88** to allow system liquid to be pumped all the way through valve **18**, holding loop **16**, switching valve **3**, input channel **4** and probe **7** into wash station **10**, removing all of the remaining cleaning liquid. The actual amount of time needed may vary depending on the system being used and the length of the channels. The amount of Y2 seconds used in step **88** may be the same or different from the amount of time used in step **75** for wash cycle **68** on branch **5**. One of ordinary skill in the art, guided by the disclosure provided herein, can determine how much time will be required to wash out the cleaning liquids.

[0061] Wash pump **32** is turned off in step **89** and control valve **18** is switched to connect pump channel **23** and holding loop **16** in step **90**. In step **91**, pump flow control valve **28** is switched to connect pump channel **23** and pump **30**. All fluid is then expelled out of pump **30** in step **92**. Step **93** shows the completion of wash cycle **81**.

[0062] FIG. 4 shows another embodiment of the present invention and is intended to be non-limiting in its scope. In FIG. 4, a sequence of steps to add reagent on both branches of the system is described. Reagent addition cycle **94** starts by switching control valve **17** to connect holding loop **15** and pump channel **19** in step **95**. Then in step **96**, pump flow control valve **27** is switched to connect pump channel **19** and pump **29**. The probe **7** is then moved to a user selected reagent vial **11** in step **97**. A volume of reagent is aspirated into probe **7** and input channel **4** by issuing an aspirate command to pump **29** in step **98**. The volume of reagent aspirated into the probe and probe **7** and input channel **4** is selected by the user of the system and may be any amount necessary to complete the relevant tests.

[0063] The probe **7** is then moved from the reagent vial into the sample container of the next sample to be loaded in step **99**. The reagent is then dispensed into sample from input channel **4** by issuing a dispense command to pump **29** in step **100**. Reagent addition cycle **94** is shown as completed in step **101**.

[0064] Reagent addition cycle **102** for branch **6** of the system is the same as reagent addition cycle **94** for branch **5** with all elements of branch **5** replaced with corresponding elements of branch **6**. The reagent addition cycle **102** is illustrated in steps **103** through **109**. Reagent addition cycle **102** starts by switching control valve **18** to connect holding loop **16** and pump channel **23** in step **103**. Then in step **104**, pump flow control valve **28** is switched to connect pump channel **23** and pump **30**. The probe **7** is then moved to a user selected reagent vial **11** in step **105**. A volume of reagent is aspirated into probe **7** and input channel **4** by issuing an aspirate command to pump **30** in step **106**. The volume of reagent aspirated into the probe and probe **7** and input channel **4** is selected by the user of the system and may be any amount necessary to complete the relevant tests.

[0065] The probe **7** is then moved from the reagent vial into the sample container of the next sample to be loaded in step **107**. The reagent is then dispensed into sample from input channel **4** by issuing a dispense command to pump **30** in step **108**. Reagent addition cycle **102** is shown as completed in step **109**.

[0066] FIG. 5 shows another embodiment of the present invention and is intended to be non-limiting in its scope. In FIG. 5, a sequence of steps to mix and load the next sample on both branches of the system is described. For some types of samples, such as living cells or beads that may settle at the bottom of sample containers **9**, it may be desired to mix the sample several times before aspirating it into holding loop **70**. Mix and load next sample cycle **110** begins by switching control valve **17** to connect holding loop **15** and pump channel **19** in step **111**. In step **112**, pump flow control valve **27** is switched to connect pump channel **19** and pump **29**. The probe **7** is then moved into a sample container **9** of the next sample to be loaded in step **113**. A user defined sample mix volume is aspirated into the probe **7** and input channel **4** by issuing an aspirate command to pump **29** in step **114**. The sample is then dispensed back into sample container **9** by issuing a dispense command to pump **29** in step **115**.

[0067] The system user is able to define any number of mixing cycles necessary to complete the desired testing. If the user defined number of mixing cycles has not been completed in step **116** then execution repeats from step **114**. Otherwise, a user defined sample volume and input channel dead volume is aspirated into holding loop **15**

with pump **29** in step **117**. The mix and load next sample cycle **110** is shown as completed in step **118**.

[0068] Mix and load next sample cycle **119** for branch **6** of the system is the same as mix and load next sample cycle **110** for branch **5** with all elements of branch **5** replaced with corresponding elements of branch **6**. The mix and load next sample cycle **119** is illustrated in steps **120** through **127**. Mix and load next sample cycle **119** begins by switching control valve **18** to connect holding loop **16** and pump channel **23** in step **120**. In step **121**, pump flow control valve **28** is switched to connect pump channel **23** and pump **30**. The probe **7** is then moved into a sample container **9** of the next sample to be loaded in step **122**. A user defined sample mix volume is aspirated into the probe **7** and input channel **4** by issuing an aspirate command to pump **30** in step **123**. The sample is then dispensed back into sample container **9** by issuing a dispense command to pump **30** in step **124**.

[0069] The system user is able to define any number of mixing cycles necessary to complete the desired testing. If the user defined number of mixing cycles has not been completed in step **125** then execution repeats from step **123**. Otherwise, a user defined sample volume and input channel dead volume is aspirated into holding loop **16** with pump **30** in step **126**. The mix and load next sample cycle **119** is shown as completed in step **127**.

[0070] FIGS. 6A and 6B show another embodiment of the present invention and are intended to be non-limiting in its scope. In FIG. 6A, a sequence of steps to inject the sample and wash interface line **2** on both branches of the system is described. The inject sample and wash interface line cycle begins at step **128**. After a sample is injected into the inspection instrument **1**, the time in which it takes the sample to traverse interface channel **2** may be significant. This is especially true if the inspection instrument **1** is a flow cytometer, where rates can be as slow as approximately 1 μL/sec. To reduce time for sample injection, a user defined boost volume corresponding to internal volume of interface channel **2** is expelled from the holding loop **15** into inspection instrument **1** at a high speed in step **129**. For example, the rate at which the sample is injected into the inspection instrument may be as high as about 10 μL/sec or greater. In another embodiment, the rate at which the sample is injected into the inspection instrument may be as high as about 50 μL/sec or greater. In still another embodiment, the rate at which

the sample is injected into the inspection instrument may be as high as about 100 μL/sec or greater. This effectively brings the front of the sample plug into the inspection instrument **1** in a short amount of time.

[0071] The injection may be made either with pressure or without pressure. In step **130**, if a user did not select to use a pressurized sample delivery, then the sample is injected into inspection instrument **1** by dispensing a user defined sample volume with pump **29** at normal speed in step **131**. Otherwise steps **132** and **133** are executed. In step **132**, control valve **17** is switched to connect holding loop **15** and pressurized channel **22**. The user may specify a delay sufficient to inject the sample into the inspection instrument in step **133**. In step **134**, control valve **17** is switched to connect holding loop **15** and waste channel **21**. The user may specify a cleaning delay in step **135** to allow back pressure from inspection instrument **1** to backwash interface channel **2** into the waste reservoir **13**. Inject sample and wash interface line cycle **128** is completed as shown in step **136**.

[0072] FIG. 6B shows inject sample and wash interface line cycle **137** for branch **6** of the system and is the same as inject sample and wash interface line cycle **128** for branch **5** with all elements of branch **5** replaced with corresponding elements of branch **6**. The inject sample and wash interface line cycle **137** is illustrated in steps **138** through **145**. A user defined boost volume corresponding to internal volume of interface channel **2** is expelled from the holding loop **16** into inspection instrument **1** at a high speed in step **138**. For example, the rate at which the sample is injected into the inspection instrument may be as high as about 10 μL/sec or greater. In another embodiment, the rate at which the sample is injected into the inspection instrument may be as high as about 50 μL/sec or greater. In still another embodiment, the rate at which the sample is injected into the inspection instrument may be as high as about 100 μL/sec or greater. This effectively brings the front of the sample plug into the inspection instrument **1** in a short amount of time.

[0073] The injection may be made either with pressure or without pressure. In step **139**, if a user did not select to use a pressurized sample delivery, then the sample is injected into inspection instrument **1** by dispensing a user defined sample volume with pump **30** at normal speed in step **140**. Otherwise steps **141** and **142** are executed. In step **141**, control valve **18** is switched to connect holding loop **16** and pressurized channel **26**.

The user may specify a delay sufficient to inject the sample into the inspection instrument in step **142**. In step **143**, control valve **18** is switched to connect holding loop **16** and waste channel **25**. The user may specify a cleaning delay in step **144** to allow back pressure from inspection instrument **1** to backwash interface channel **2** into the waste reservoir **13**. Inject sample and wash interface line cycle **137** is completed as shown in step **145**.

[0074] The high-speed sample supply device disclosed herein is useful to deliver samples to any type of inspection instrument. Preferably, the device disclosed herein is used to delivery samples to a flow cytometer. Flow cytometers are well-known analytical tools that are able to analyze several thousand particles every second and can actively separate and isolate particles having specified properties. For example, the high-speed sample supply device may be used in flow cytometers described in U.S. Patent Nos. 6,713,019; 5,824,269; 5,367,474; 5,135,502; and 4,702,598; all of which are hereby incorporated by reference in their entirety.

[0075] All patents incorporated by reference herein are incorporated by reference herein only with respect to the particular embodiments, materials, processes of manufacture and methods of use described therein. These patent are not to be considered incorporated by reference to the extent any of these patents expresses an opinion or presents any representation, characterization, or definition (either expressly or by implication) that is inconsistent with the opinions, representations, characterizations or definitions expressly made herein.

[0076] While there have been described herein what are to be considered exemplary and preferred embodiments of the present invention, other modifications of the invention will become apparent to those skilled in the art from the teachings herein. It is therefore desired to be secured in the appended claims all such modifications as fall within the true spirit and scope of the invention. Accordingly, what is desired to be secured by Letters Patent is the invention as defined and differentiated in the following claims.

WHAT IS CLAIMED IS:

1. A method for multiplexed sample analysis, comprising:

(a) robotically obtaining a first liquid sample from the first of a plurality of sample sources;

(b) delivering the first liquid sample through a first line to an instrument for analysis of the sample;

(c) robotically obtaining a second liquid sample from the second of a plurality of sample sources;

(d) delivering the second liquid sample through a second line to an instrument for analysis;

(e) cleaning the second line while the first line is delivering sample to the instrument; and

(f) cleaning the first line while the second line is delivering sample to the instrument.

2. The method of Claim 1, further comprising:

(g) repeating steps (a)-(f) to obtain and deliver multiple samples to the instrument from all of the plurality of sample sources.

3. The method of Claim 1, wherein the instrument is a flow cytometer.

4. The method of Claim 1, wherein the first and second liquid samples are independently selected from the group consisting of chemical compounds, antibodies, beads, live cells, or fixed cells.

5. The method of Claim 1, further comprising the steps of:

delivering a first reagent to the first liquid sample before the sample is delivered to the instrument; and

delivering a second reagent to the second liquid sample before the sample is delivered to the instrument.

6. The method of Claim 5, wherein delivery of the first and second reagents is performed with a separate auto-sampler arm.

7. The method of Claim 5, wherein the first and second reagents are independently selected from the group consisting of chemical compounds, antibodies, beads, live cells, or fixed cells.

8. The method of Claim 5, wherein multiple reagents are added to the sample before the sample is injected into the instrument.

9. The method of Claim 1, wherein a control valve alternates delivery of cleaning fluid with the delivery of liquid sample in each of the first and second lines.

10. The method of Claim 9, wherein the control valve further provides the delivery of air pressure or system liquid pressure.

11. The method of Claim 9, wherein the control valve further provides a waste channel.

12. An apparatus for delivering samples to an instrument for analysis, comprising:

a first and a second sample delivery line, wherein the first sample delivery line comprises a first sample loading and injection branch and the second sample delivery line comprises a second sample loading and injection branch;

a fluid flow management mechanism for alternately connecting the first and second sample delivery lines to the instrument;

at least one cleaning fluid delivery line to supply cleaning fluid to the flow management mechanism, the first sample delivery line, and the second sample delivery line;

at least one sample input channel;

at least one control valve for alternately delivering cleaning fluid to the first and second sample delivery lines, so that sample and cleaning fluid alternately flow through the first and second sample delivery lines.

13. The apparatus of Claim 12, wherein the fluid flow management mechanism comprises a position switching valve.

14. The apparatus of Claim 13, wherein the position switching valve comprises a four-way two-position switching valve.

15. The apparatus of Claim 13, wherein the position switching valve switches between a first mode and a second mode.

16. The apparatus of Claim 15, wherein the first mode of the position switching valve fluidicly couples the first sample delivery line to the instrument and fluidicly couples the second sample delivery line to the sample input channel.

17. The apparatus of Claim 15, wherein the second mode of the position switching valve fluidicly couples the first sample delivery line to the sample input channel and fluidicly couples the second sample delivery line to the instrument.

18. The apparatus of Claim 12, wherein the sample loading and injection branches of the first and second sample delivery lines comprise symmetrical sample loading and injection branches.

19. The apparatus of Claim 12, wherein the first sample delivery line comprises a first sample holding loop and the second sample delivery line comprises a second sample holding loop.

20. The apparatus of Claim 19, comprising a first control valve located within the first sample delivery line and a second control valve located within the second sample delivery line.

21. The apparatus of Claim 20, wherein the first and second control valves each comprise four fluidic channels capable of coupling the first and second sample holding loops to one of the four fluidic channels.

22. The apparatus of Claim 13, wherein one of the four fluidic channels is connected to output ports of pump flow control valves.

23. The apparatus of Claim 13, wherein one of the four fluidic channels is connected to the at least one cleaning fluid delivery line.

24. The apparatus of Claim 13, wherein one of the four fluidic channels is connected to waste disposal.

25. The apparatus of Claim 13, wherein one of the four fluidic channels is connected to a controlled air pressure source.

26. The apparatus of Claim 12, wherein the at least one sample input channel is coupled to a robotic sampler positioning device.

27. The apparatus of Claim 26, wherein the positioning device comprises one or two arms.

28. The apparatus of Claim 27, wherein the positioning device comprises two arms and the second arm comprises a reagent transfer pump.

29. The apparatus of Claim 28, wherein the reagent transfer pump comprises a syringe pump, a peristaltic pump, or a diaphragm pump.

30. The apparatus of Claim 12, wherein the instrument comprises a flow cytometer.