



I-Class SM-FL Fluidics Overview



Rev. 3.0

5/1/2013



Global Training
& Development

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I-Class SM-FL Fluidics

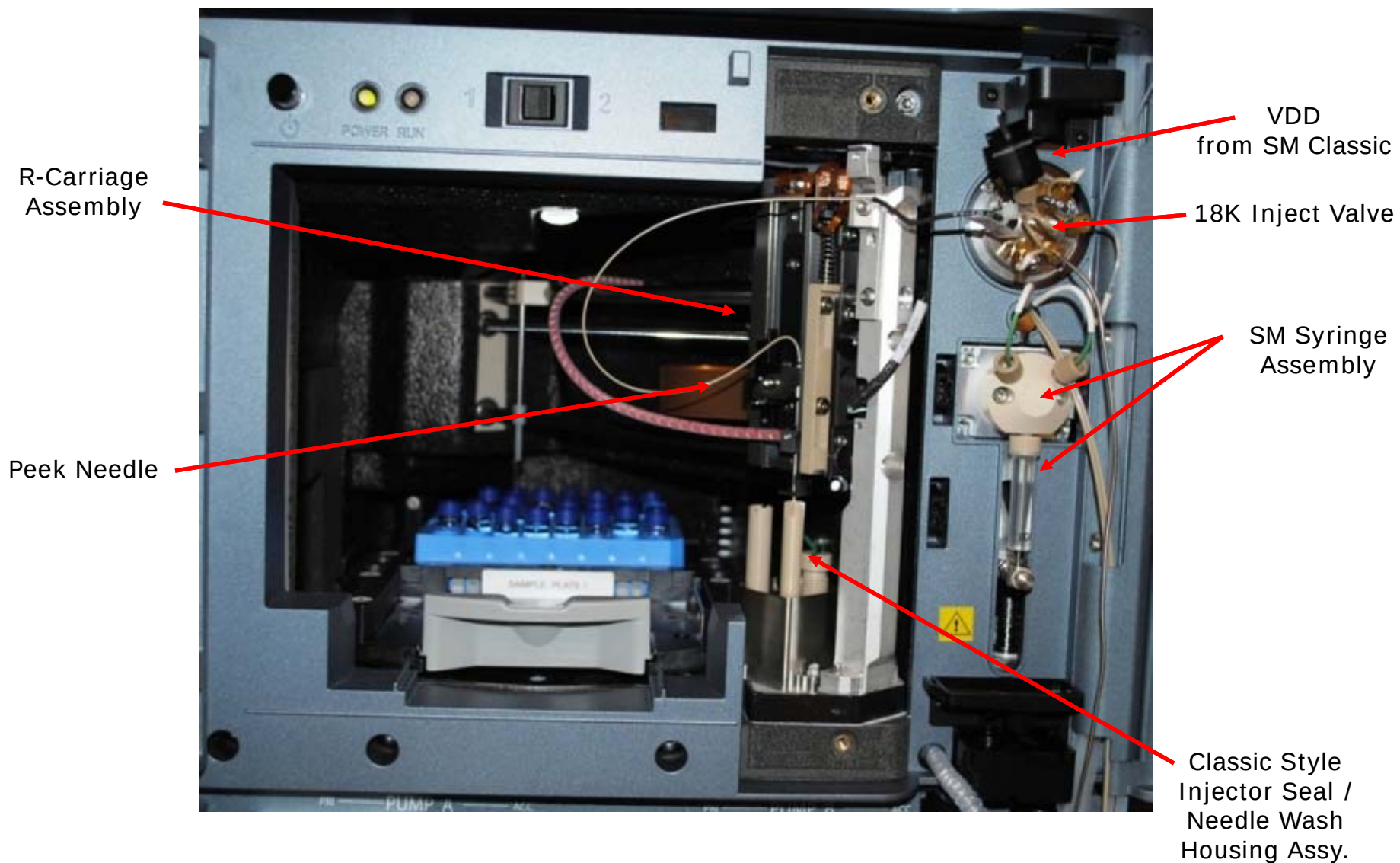


SM-FL Overview



- Implementation of the ACQUITY Classic fixed loop injection technique on the new Common Platform SM
- Most parts not specific to fixed loop injection and post injection washing are the same as the SM_FTN
- 18K psi capable
- Lower dispersion
- Reduced carryover
- New Wash Syringe drive
- Updated valve manifold

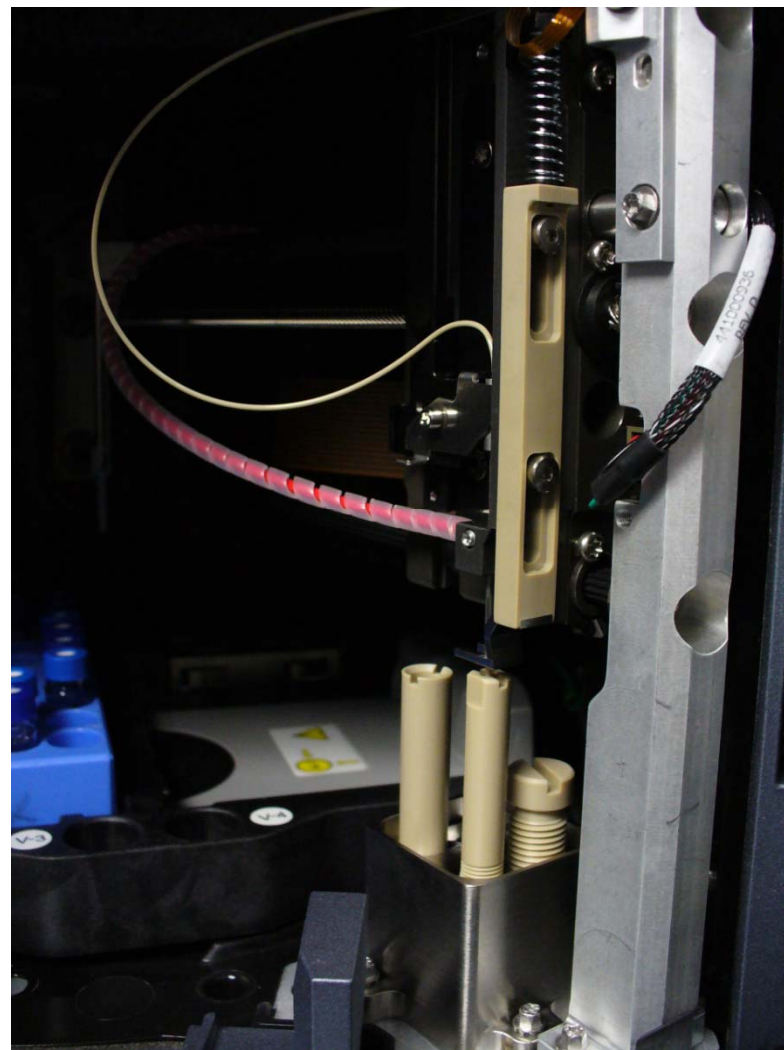
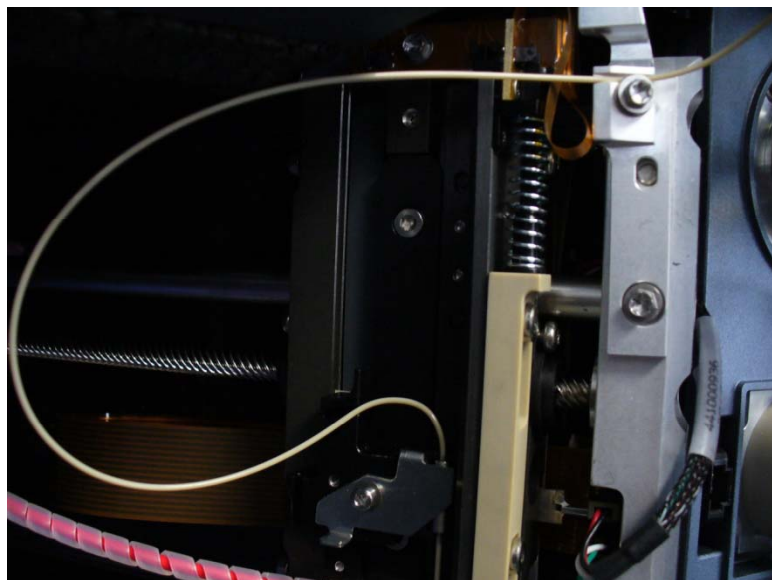
SM-FL (Fixed Loop)- I-Class Only



SM-FL: Injection Port

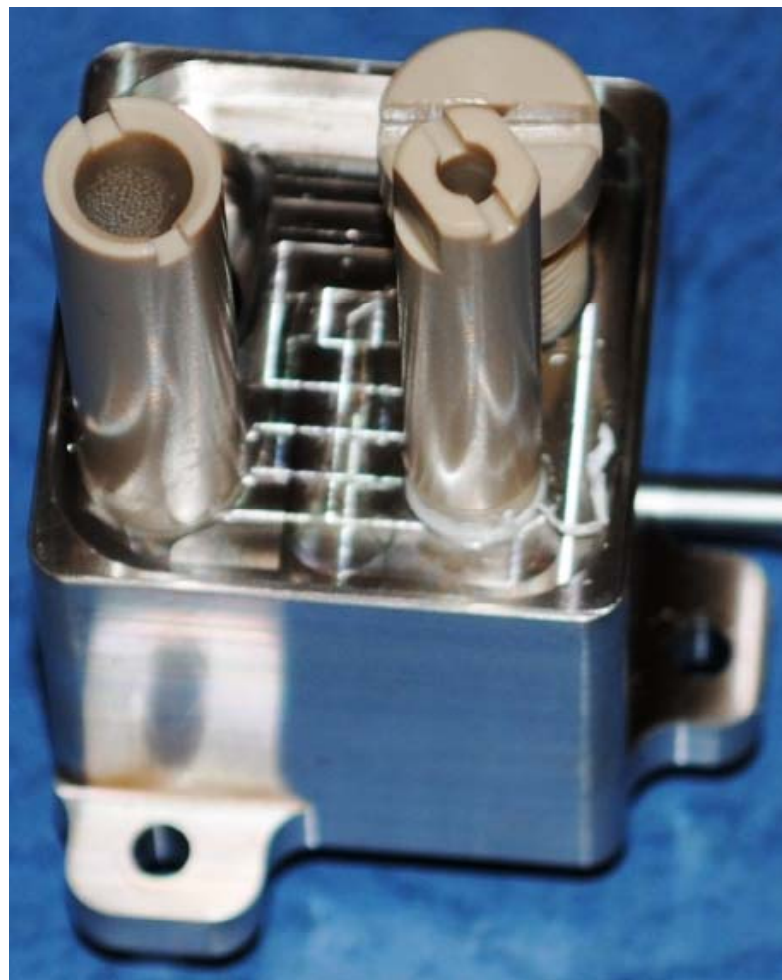
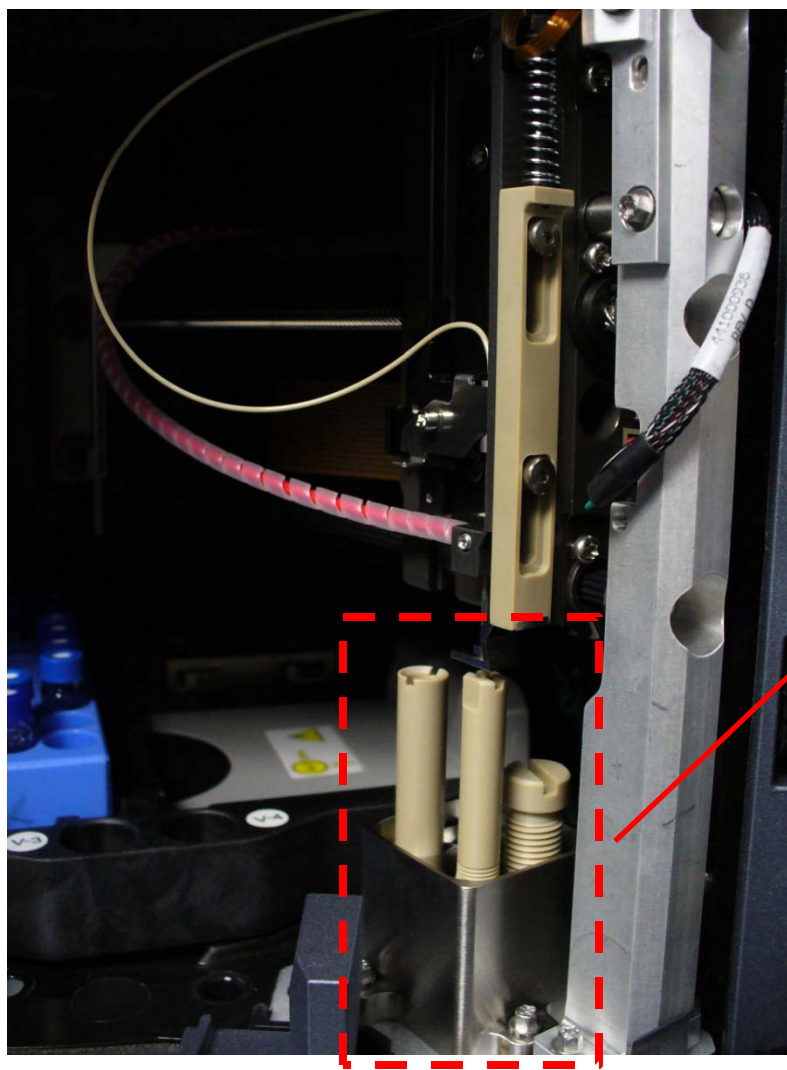


- Injection port moved to front of SM-FL
- Needle volume reduced
- New routing of needle in sample manager



SM-FL: Injection Port

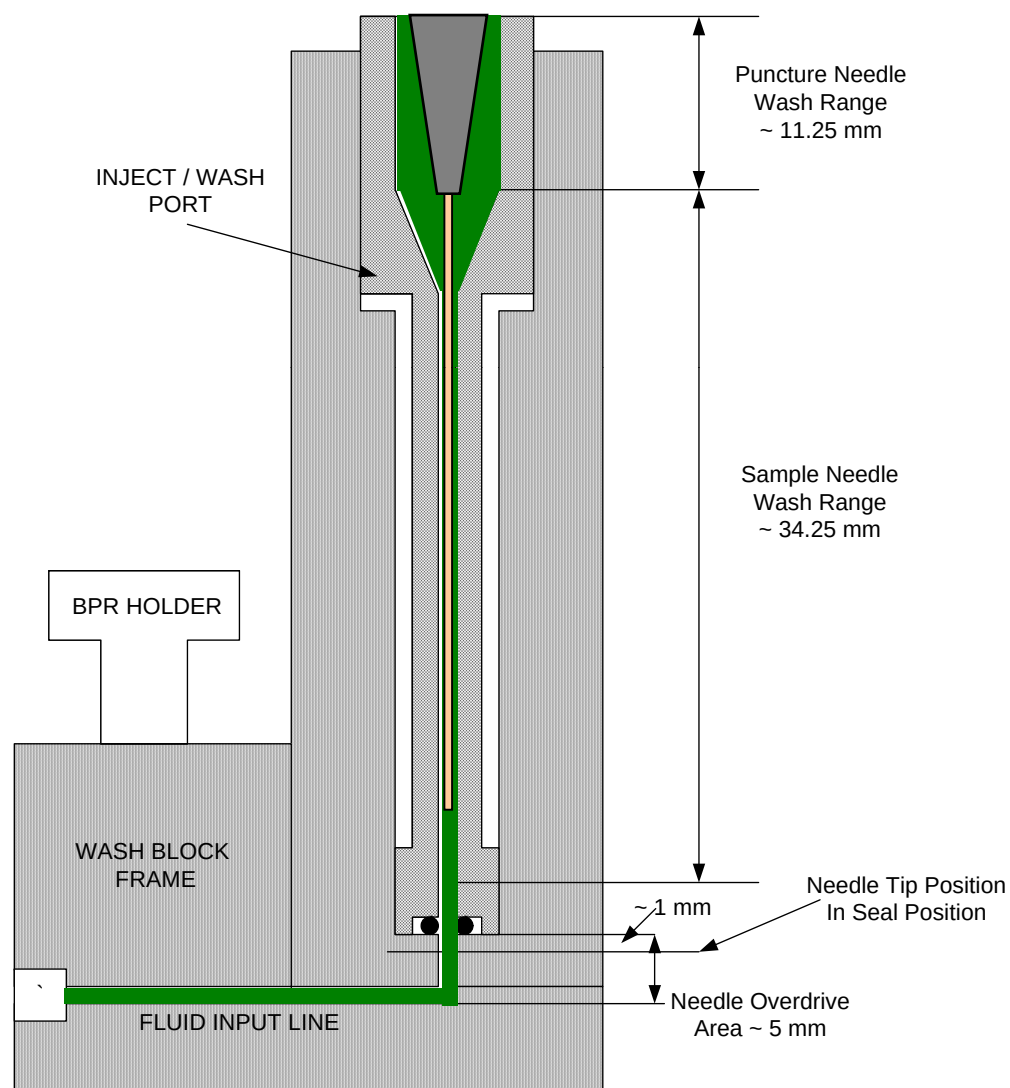
Same function different position.



Wash Block- Needle Wash Function



Wash / Inject Block Internal Detail

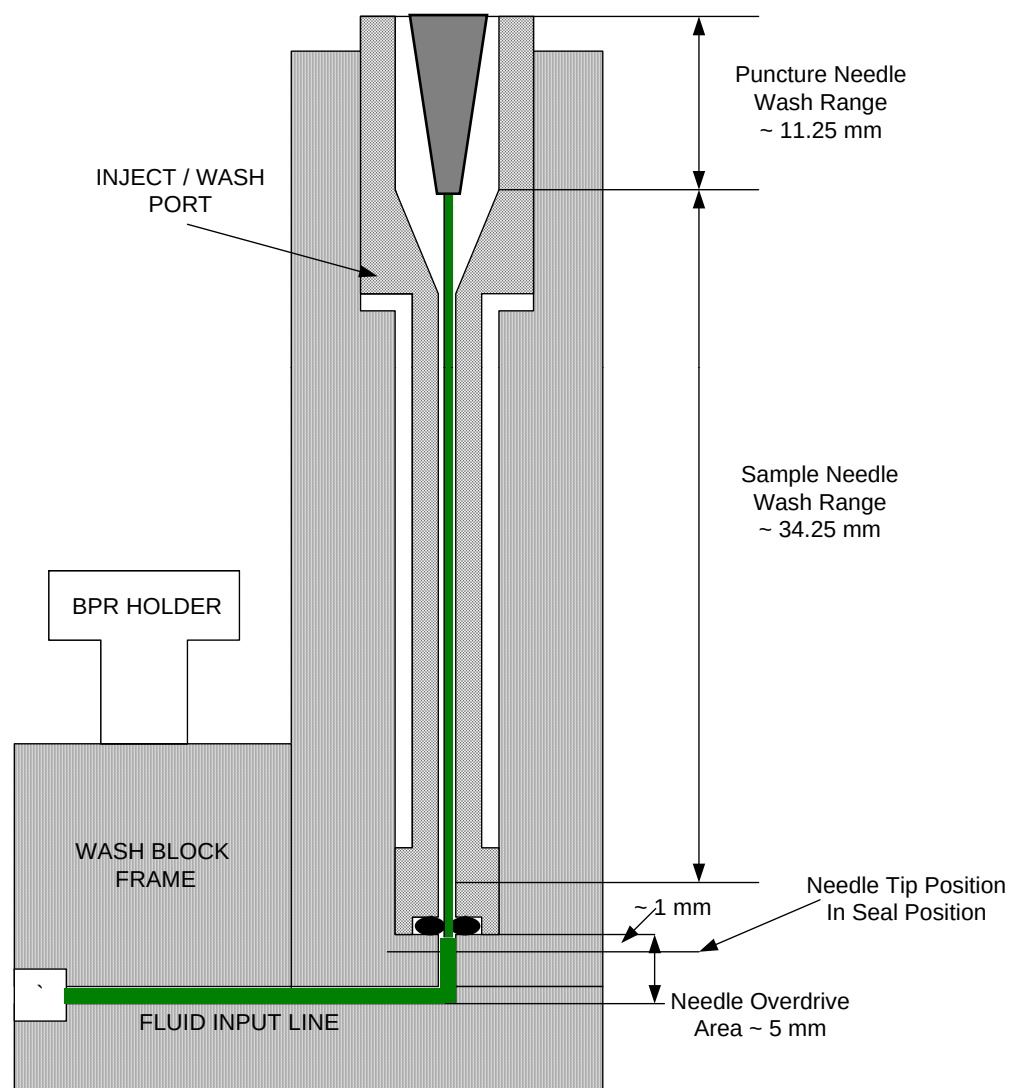


WASH / INJECT BLOCK

Wash Block- Pressure Assist Function

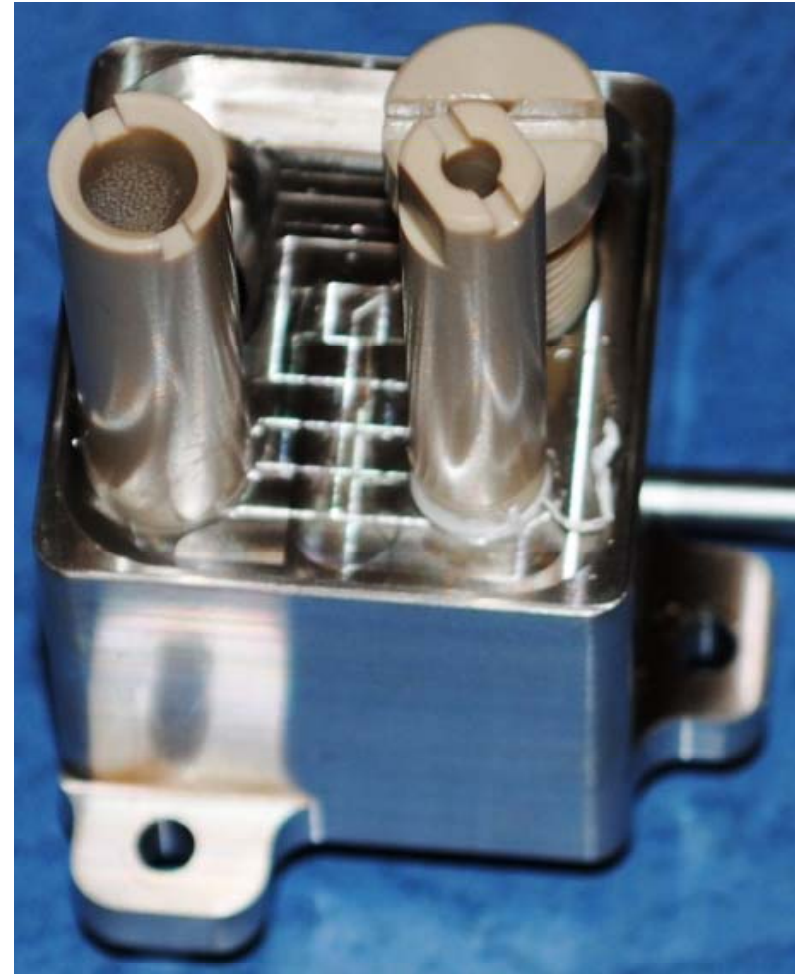
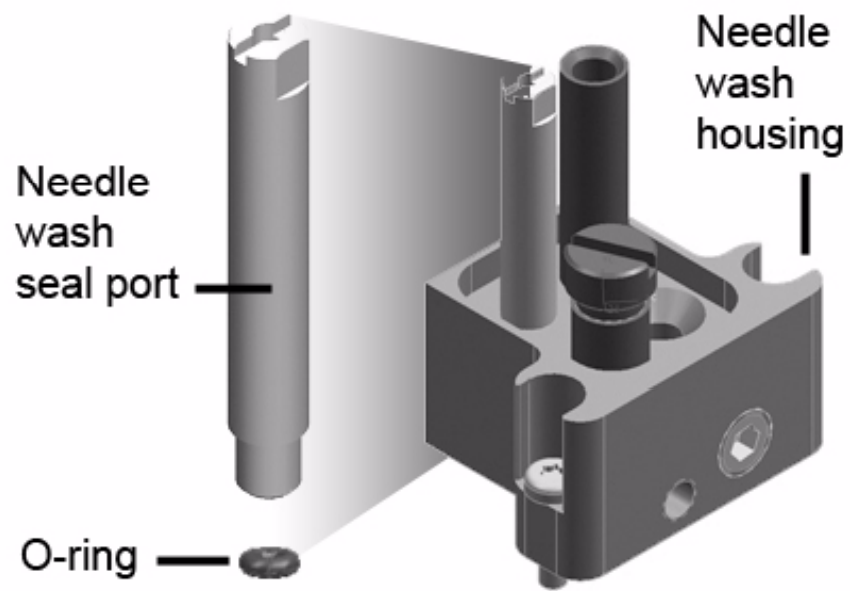


Wash / Inject Block Internal Detail



WASH / INJECT BLOCK

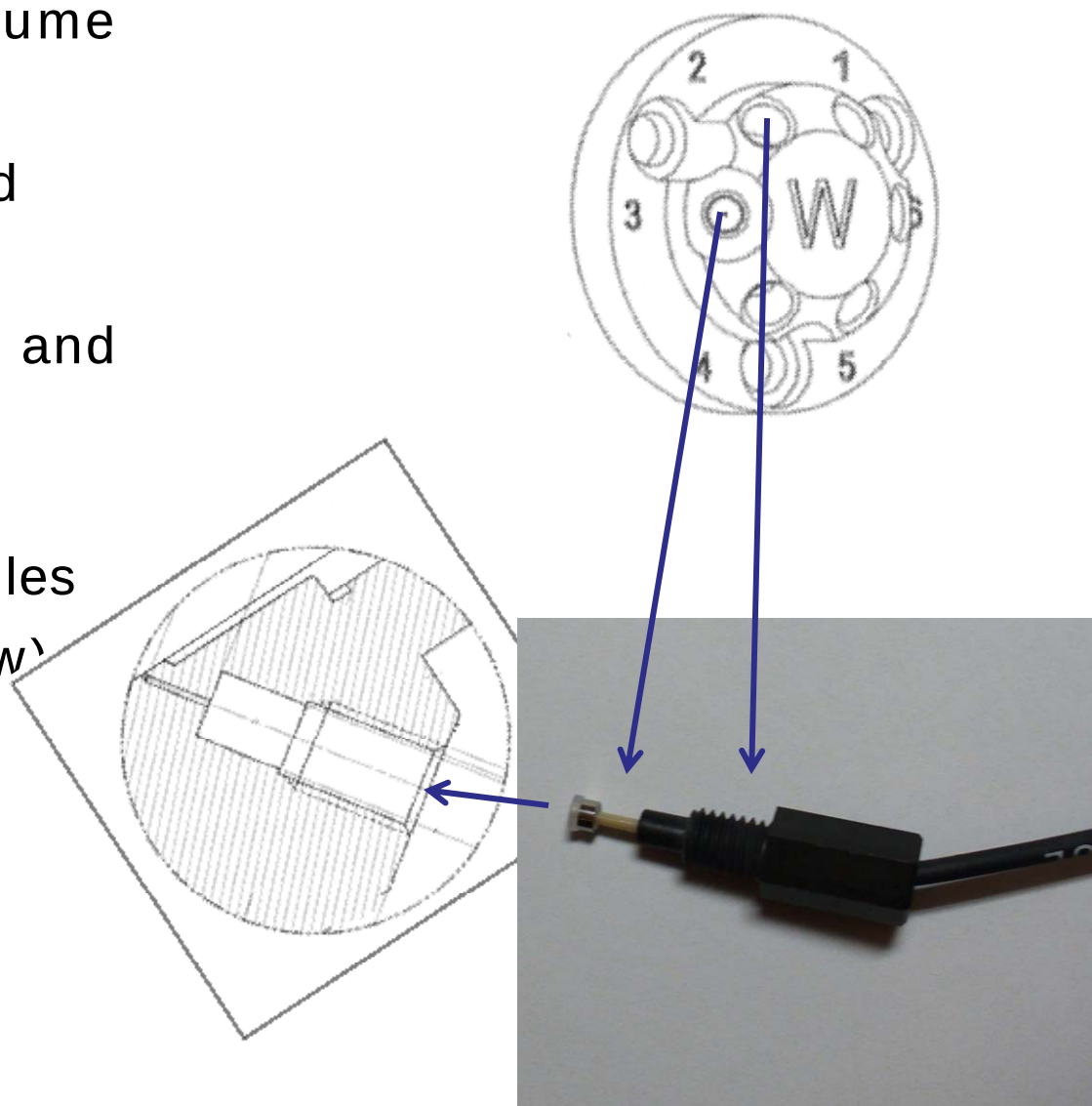
Seal Port O-Ring Replacement



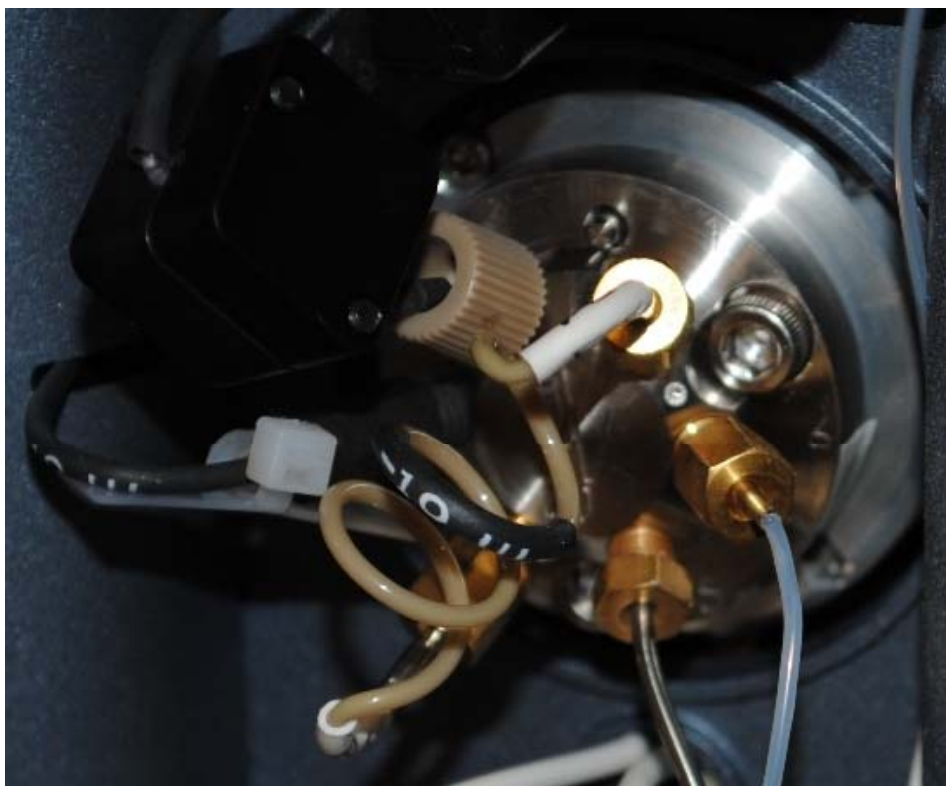
SM-FL: New Sample Needle



- New default needle volume
- Needle volume reduced
- New fittings for Needle and VDD
- Available Sample Needles
 - PEEK (Available Now)
 - PEEKSi
 - Steel
 - FEP



SM-FL: Inject Valve

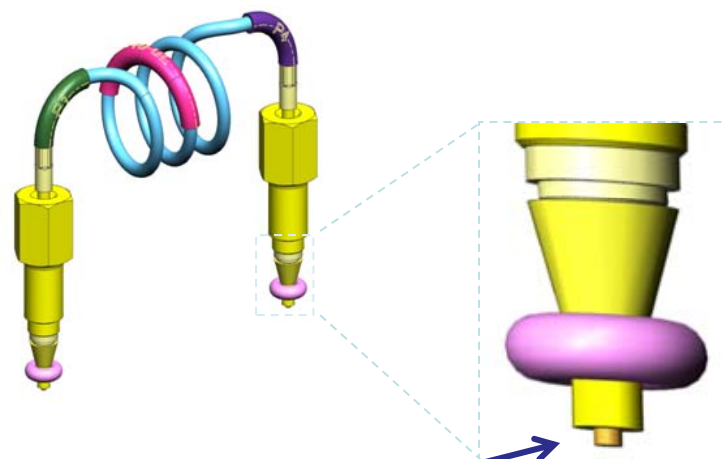


- Inject Valve
 - 18k psi compatibility
 - New rotor
 - Pod not interchangeable with ACQUITY UPLC
 - Minimized dispersion
 - Improved carryover
- New sample loops

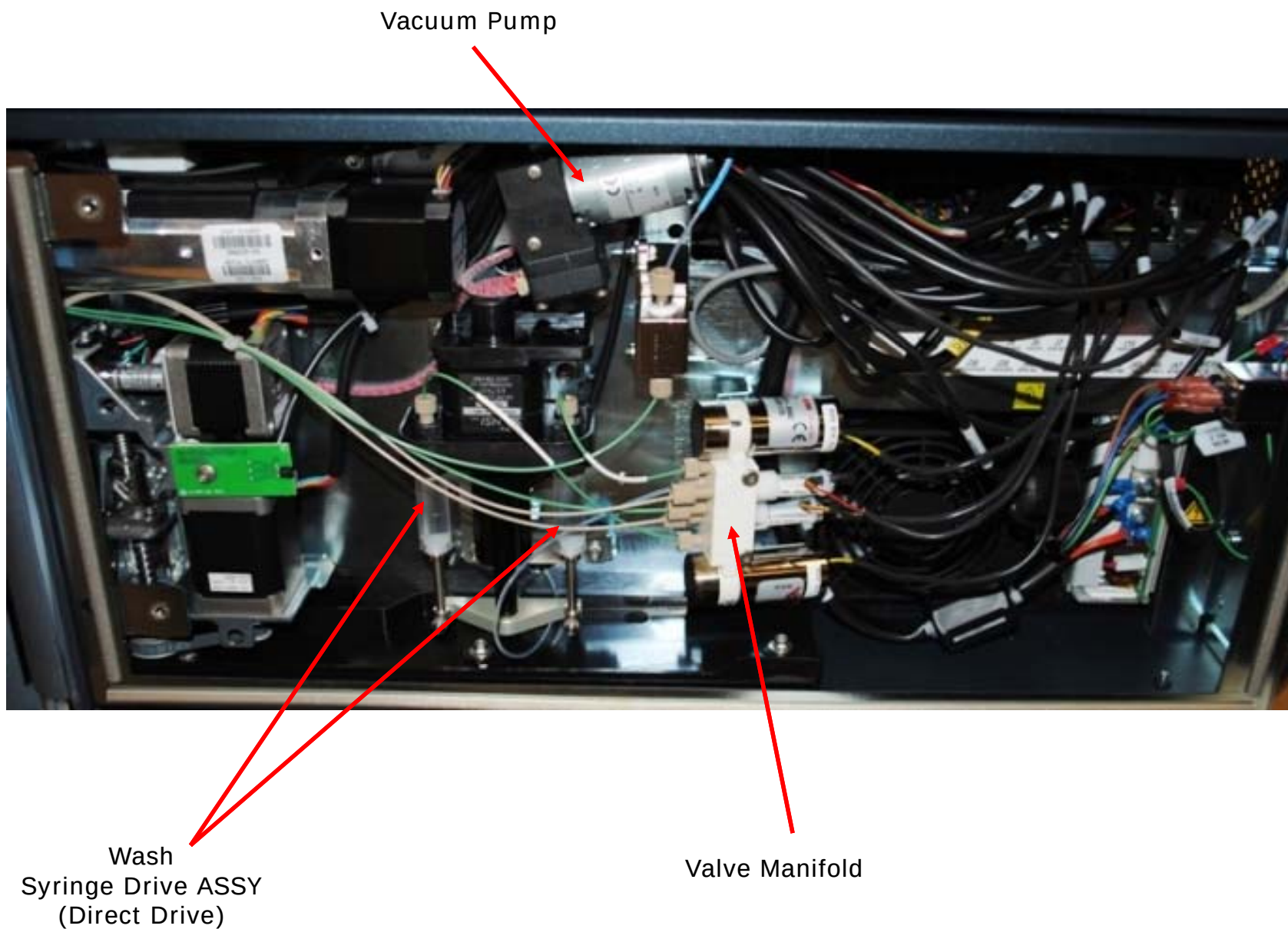
SM-FL: New Loops



- New sample loops
 - Pre-swaged to prevent poor installation
 - Smaller o.d. tubing with more consistent i.d. for better loop to loop consistency
 - Better carryover performance due to hypo fitting



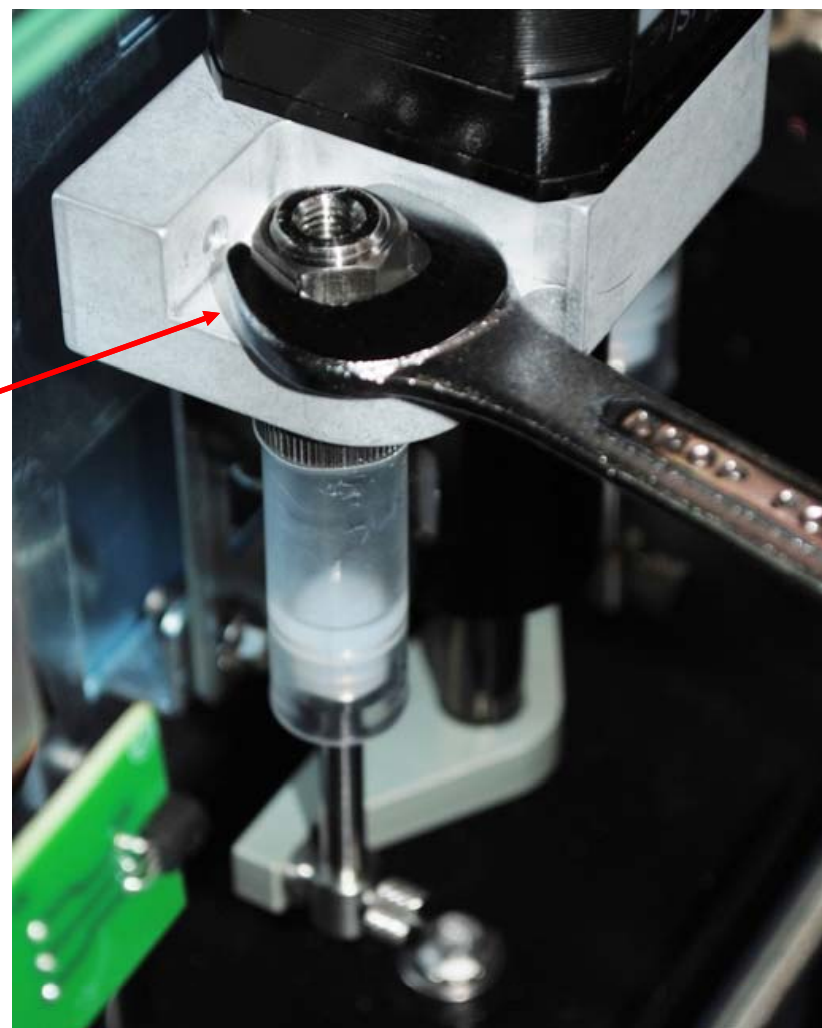
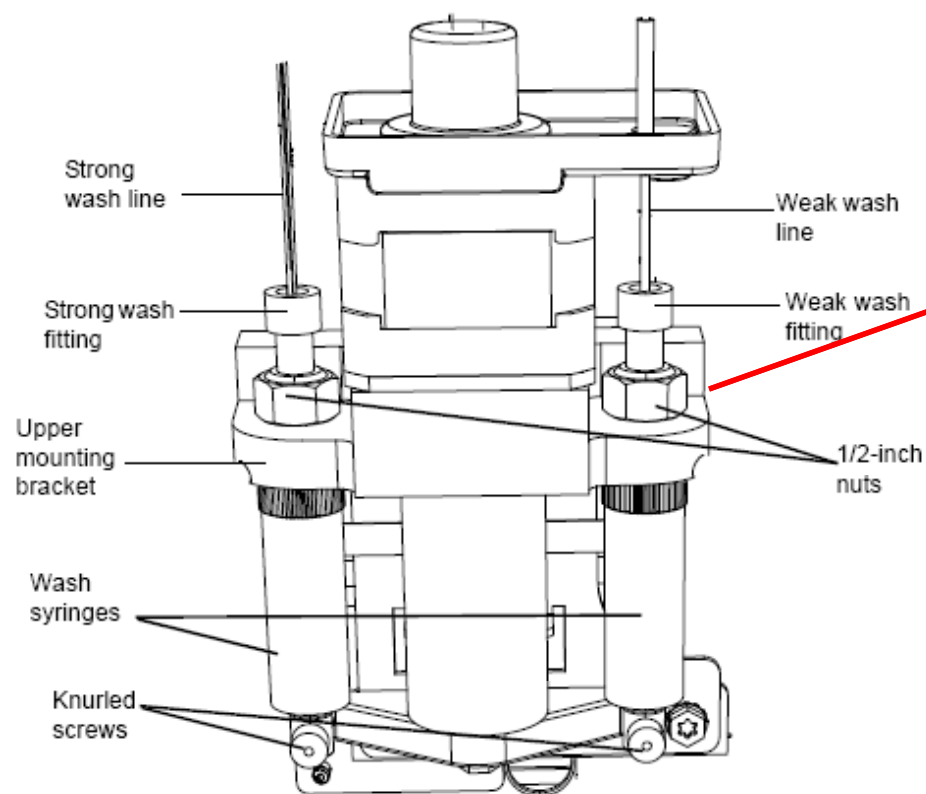
SM-FL Component Overview



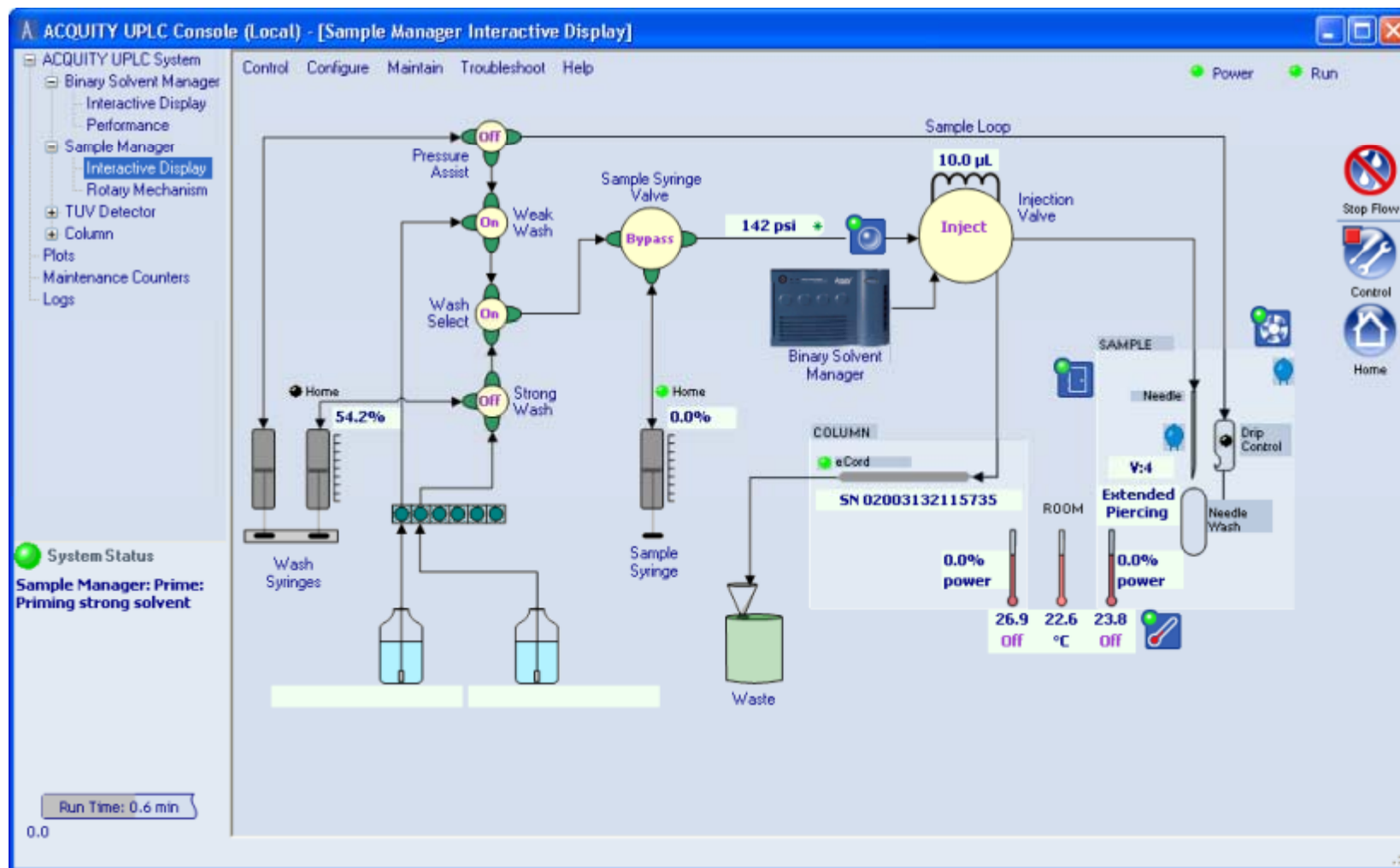
Wash Syringe Drive Assembly



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I-Class SM-FL Interactive Menu



Sample Manager Injection Methods



Sample Manager- Injection Options



- There are three injection techniques available with the ACQUITY UPLC™ Sample Manager
 - **Full Loop**
 - **Partial Loop Using Needle Overfill (PLUNO)**
 - **Partial Loop** using pressure assist

Full Loop



- Should be chosen whenever accuracy and precision are the primary concerns
- It is the same injection technique that has always existed in HPLC injectors
 - Shares all the same advantages, disadvantages, and operating constraints.
- No flexibility in injection volume without changing the loop
- Customer should design QC analysis to compensate
 - Standard same concentration as sample
 - Dilutions made to reflect constant injection volume

Partial Loop – Pressure Assist



- Should be reserved for the following situations only:
 - Where analysis time takes precedence over any other concern
 - Where the sample volume is very limited
 - When sample diluent is different than initial condition mobile phase (stronger organic content) and the diluent has a noticeable effect on the chromatography with the PLUNO and Full Loop modes

Partial Loop Using Needle Overfill Mode



- The best general purpose mode for partial loop injection
- Provides optimum performance when injection volumes are kept within a range of 20 – 75% nominal loop volume
- It provides the best partial loop accuracy, precision and linearity for a wide range of samples including
 - strong and weak acids and bases
 - hydrophilic and hydrophobic compounds.

ACQUITY UPLC™ Loops available



1 µL
2 µL
5 µL
10 µL (STD)
20 µL
50 µL
100 µL
250 µL

Full Loop and Partial Loop Mode (Both with Pressure Assist)



Full Loop and Partial Loop

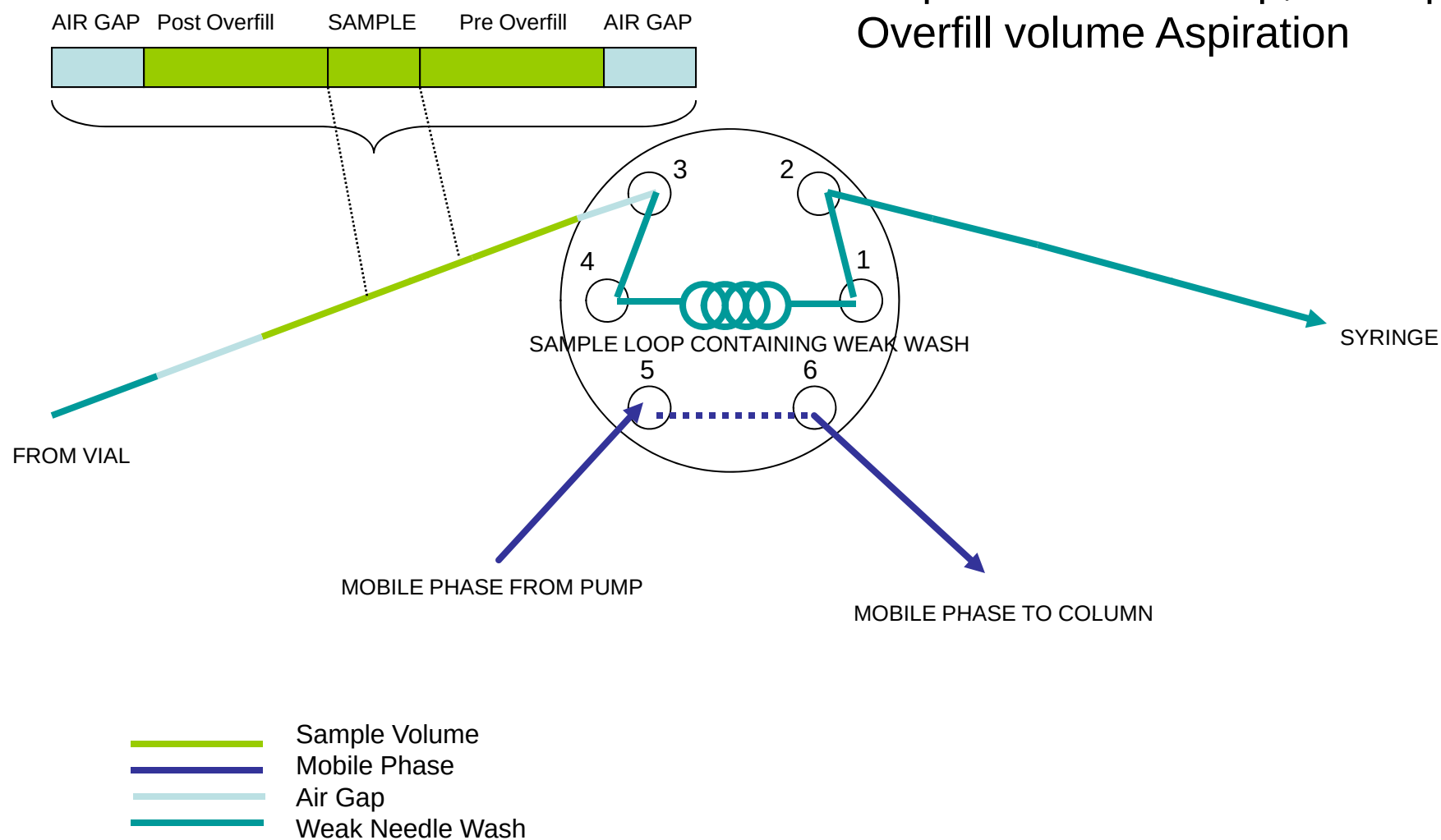


- Similarities
 - The same basic delivery mechanism to the sample loop
 - Both use Pressure Assist function to keep cycle time down
 - Both surround the sample bolus with air gaps
- Differences
 - Partial loop (PA) uses only the amount of sample programmed into the injection volume field in the sample set (or sample list)
 - Air gaps are injected unto the column
 - Full loop uses extra sample to flush the loop before the loop is filled with sample and ignores the injection volume field
 - Air gaps are not injected unto the column

Full Loop Injection Sequence - Step 1



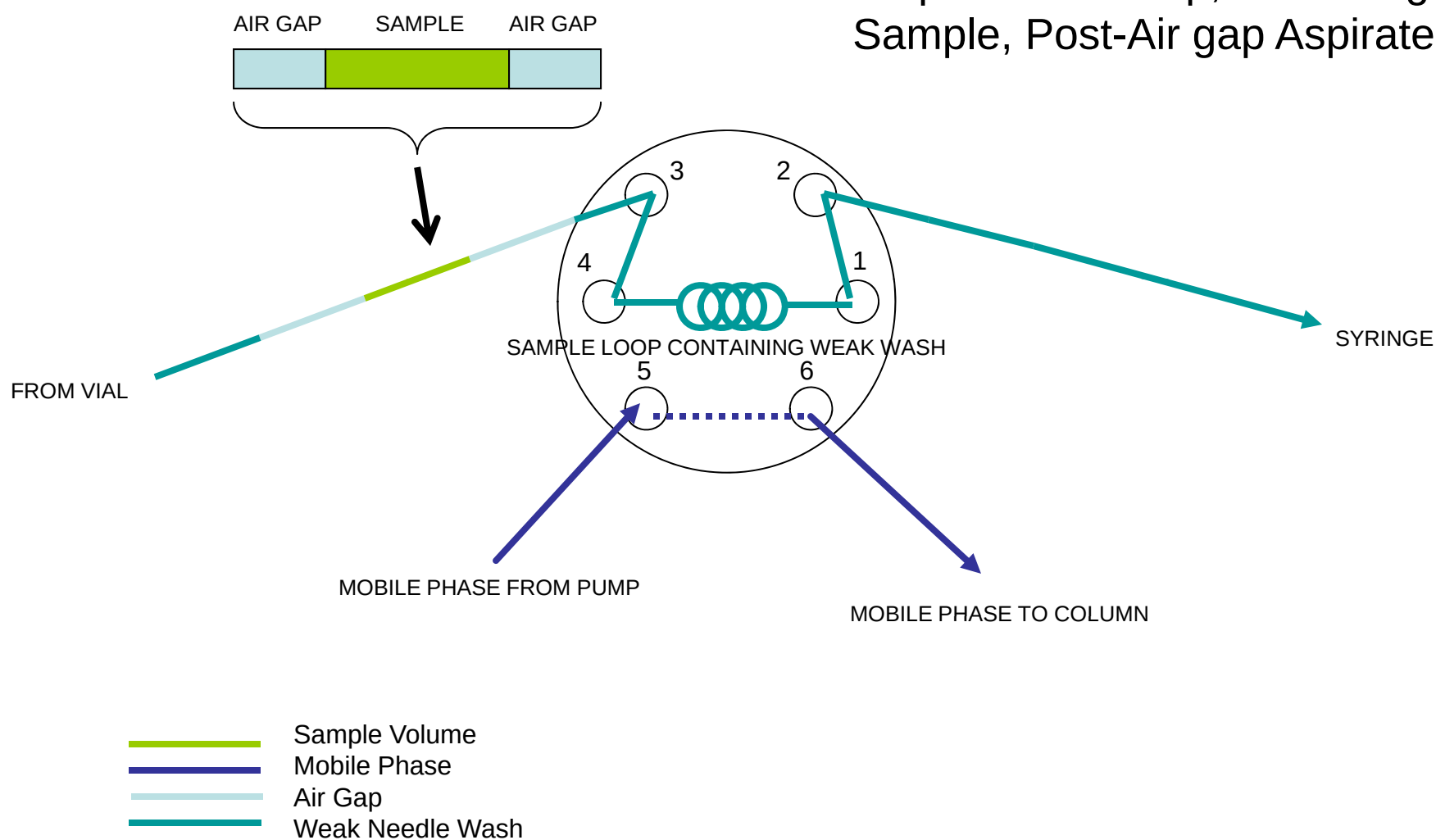
Step 1 : Air Gap, Sample, Overfill volume Aspiration



Pressure Assist Injection Sequence - Step 1



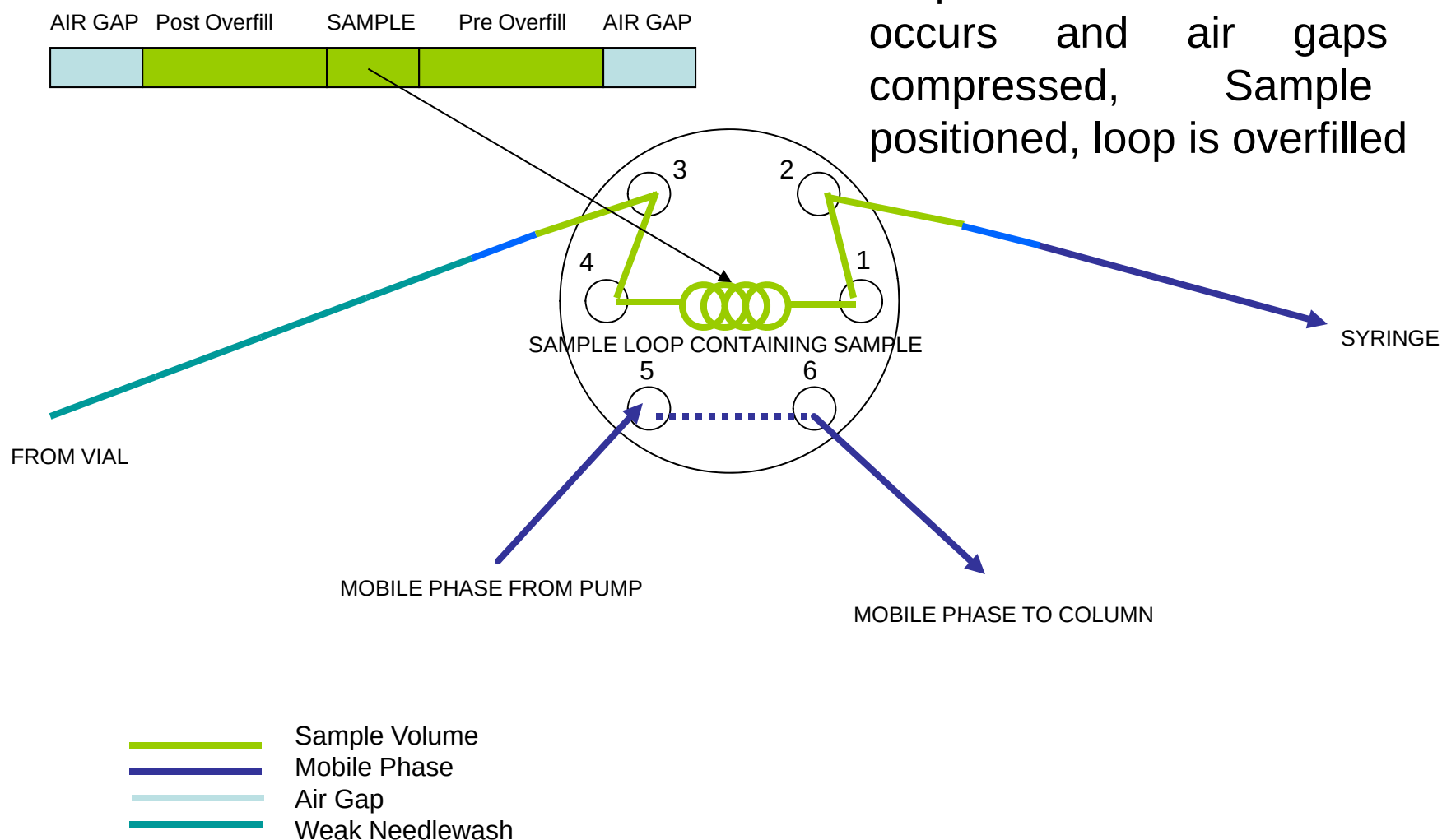
Step 1 : Air Gap, Pre-Air gap, Sample, Post-Air gap Aspirated.



Full Loop Injection Sequence - Step 2



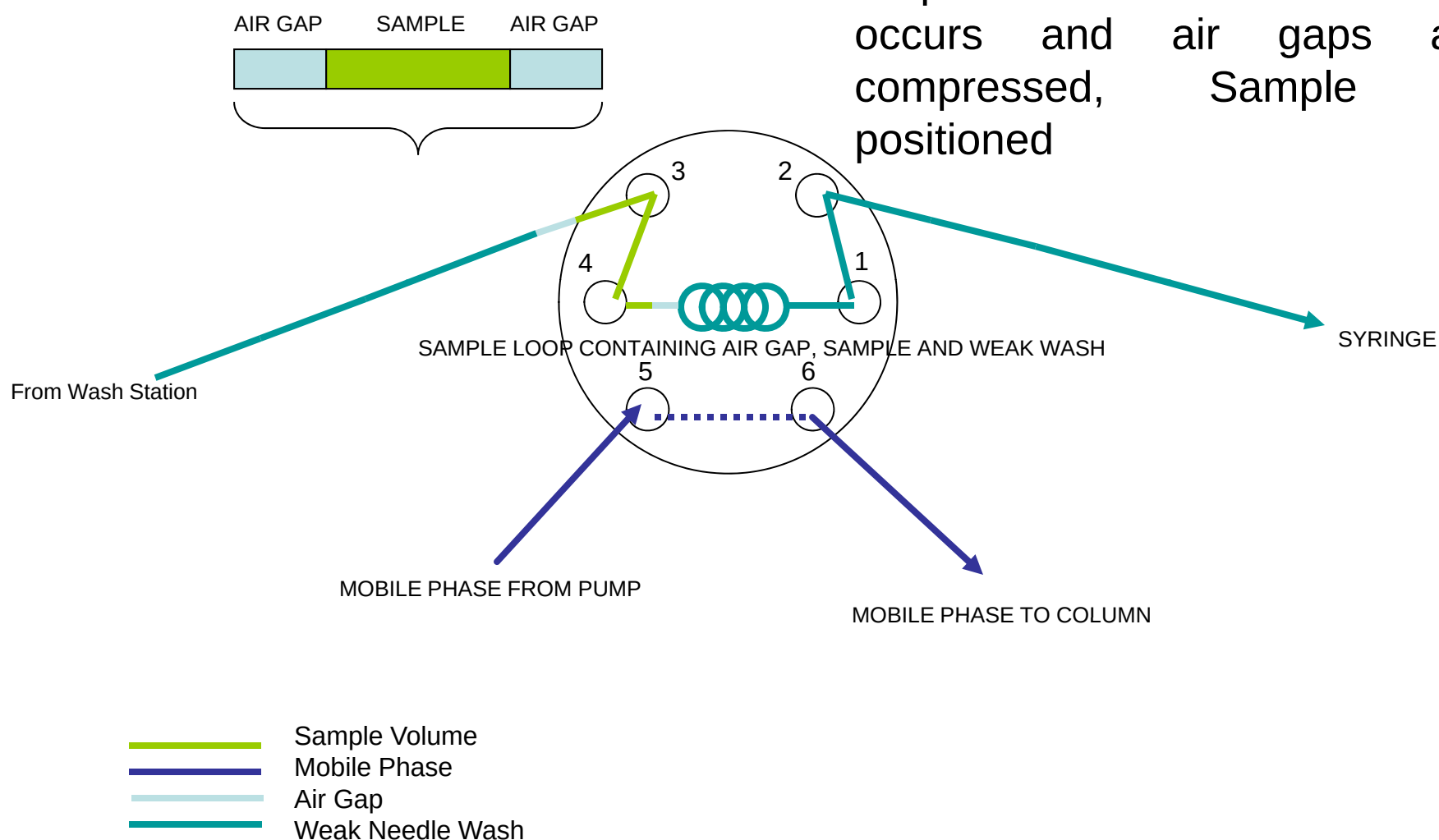
Step 1 : Pressurization to 150psi occurs and air gaps are compressed, Sample is positioned, loop is overfilled



Pressure Assist Injection Sequence - Step 2



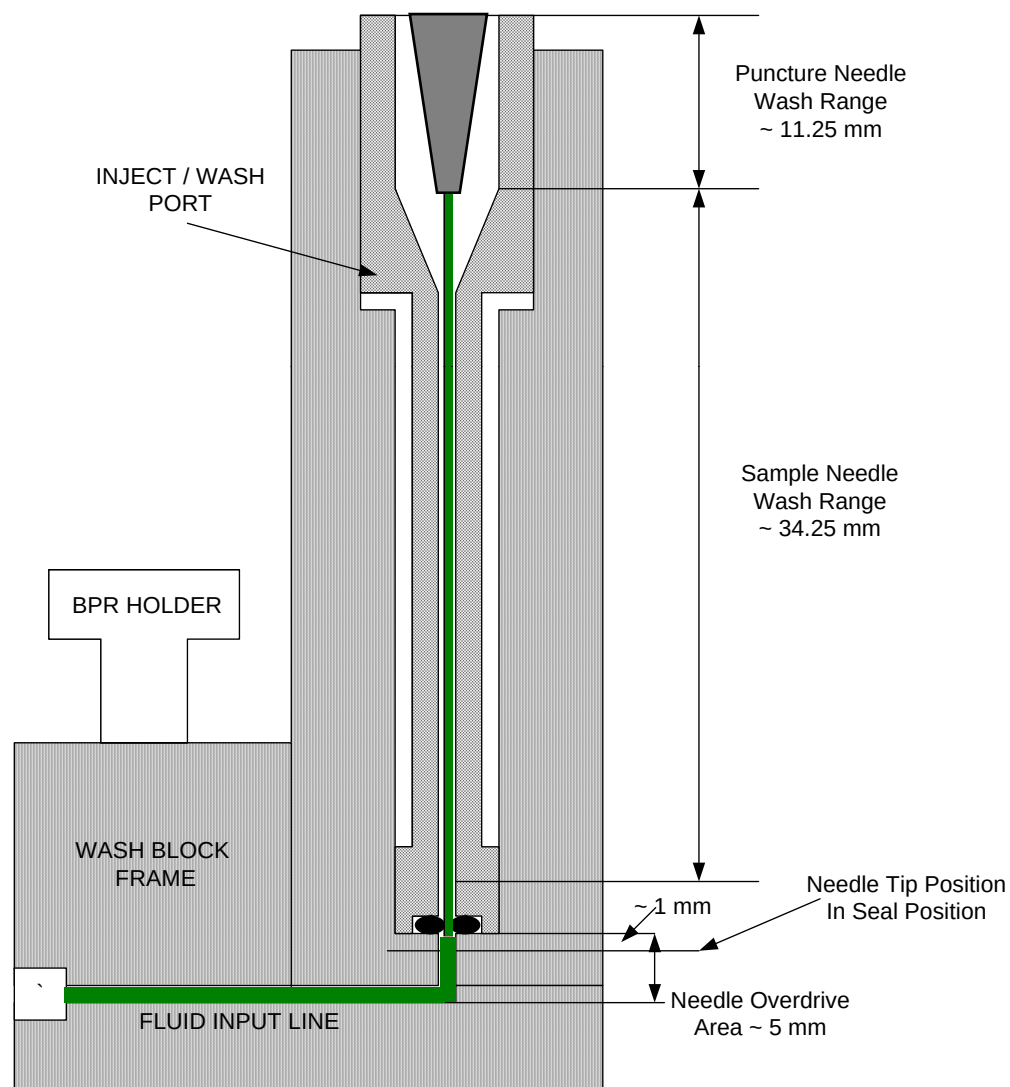
Step 2 : Pressurization to 150psi occurs and air gaps are compressed, Sample is positioned



Step 2-Pressure Assist Function

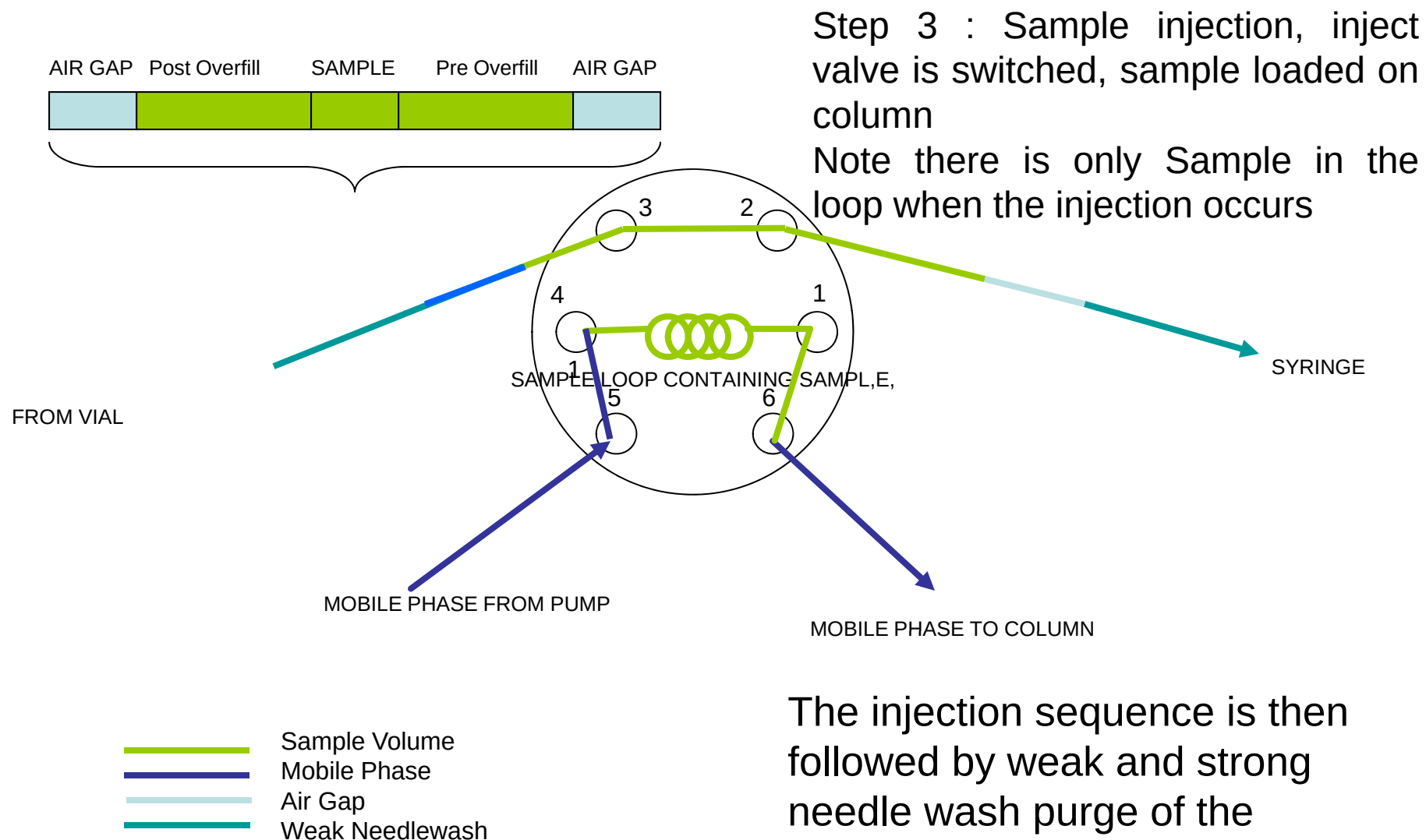


Wash / Inject Block Internal Detail



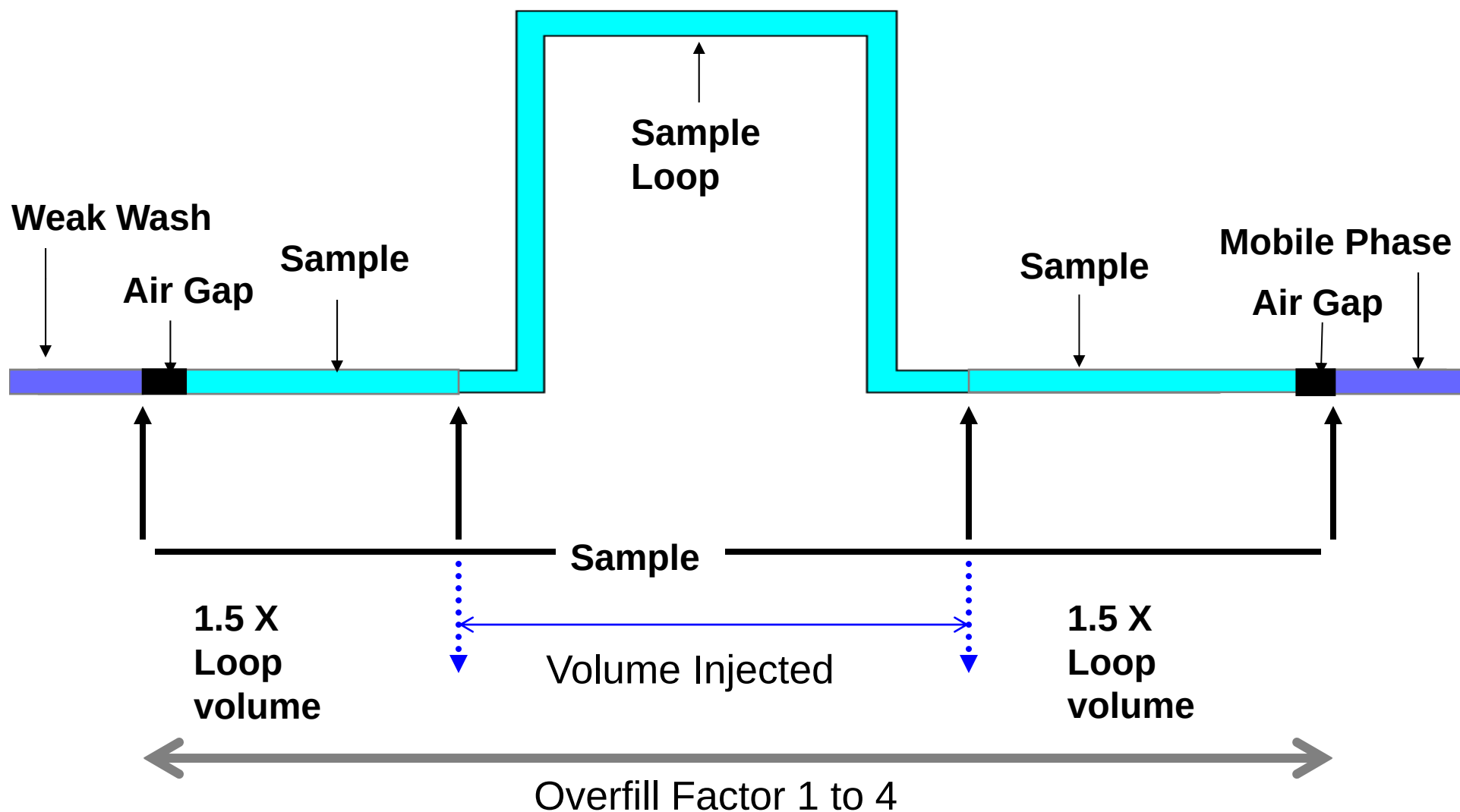
WASH / INJECT BLOCK

Full Loop Injection Sequence - Step 3

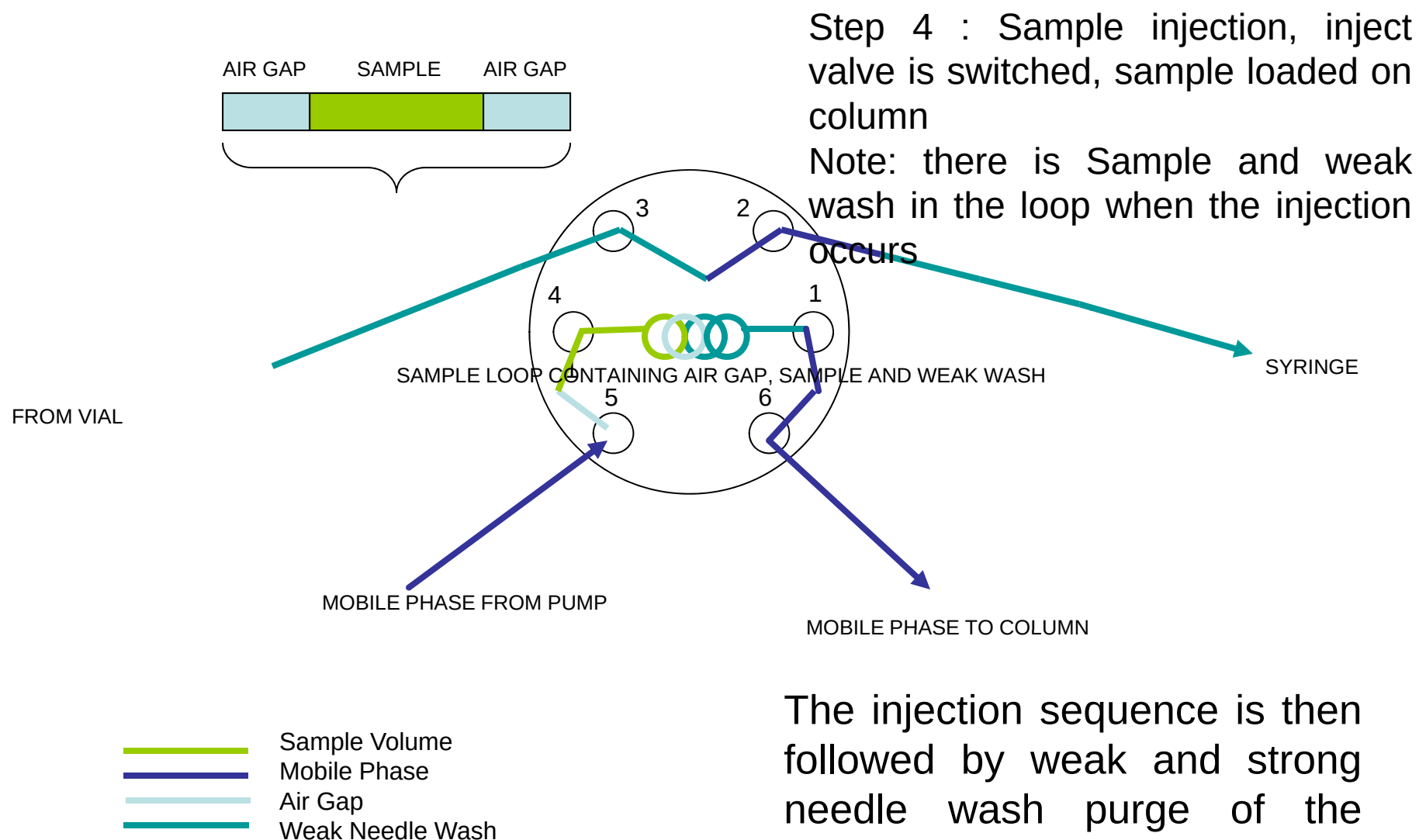


The injection sequence is then followed by weak and strong needle wash purge of the entire sample manager fluidics

Injection Loop Contents- Full Loop

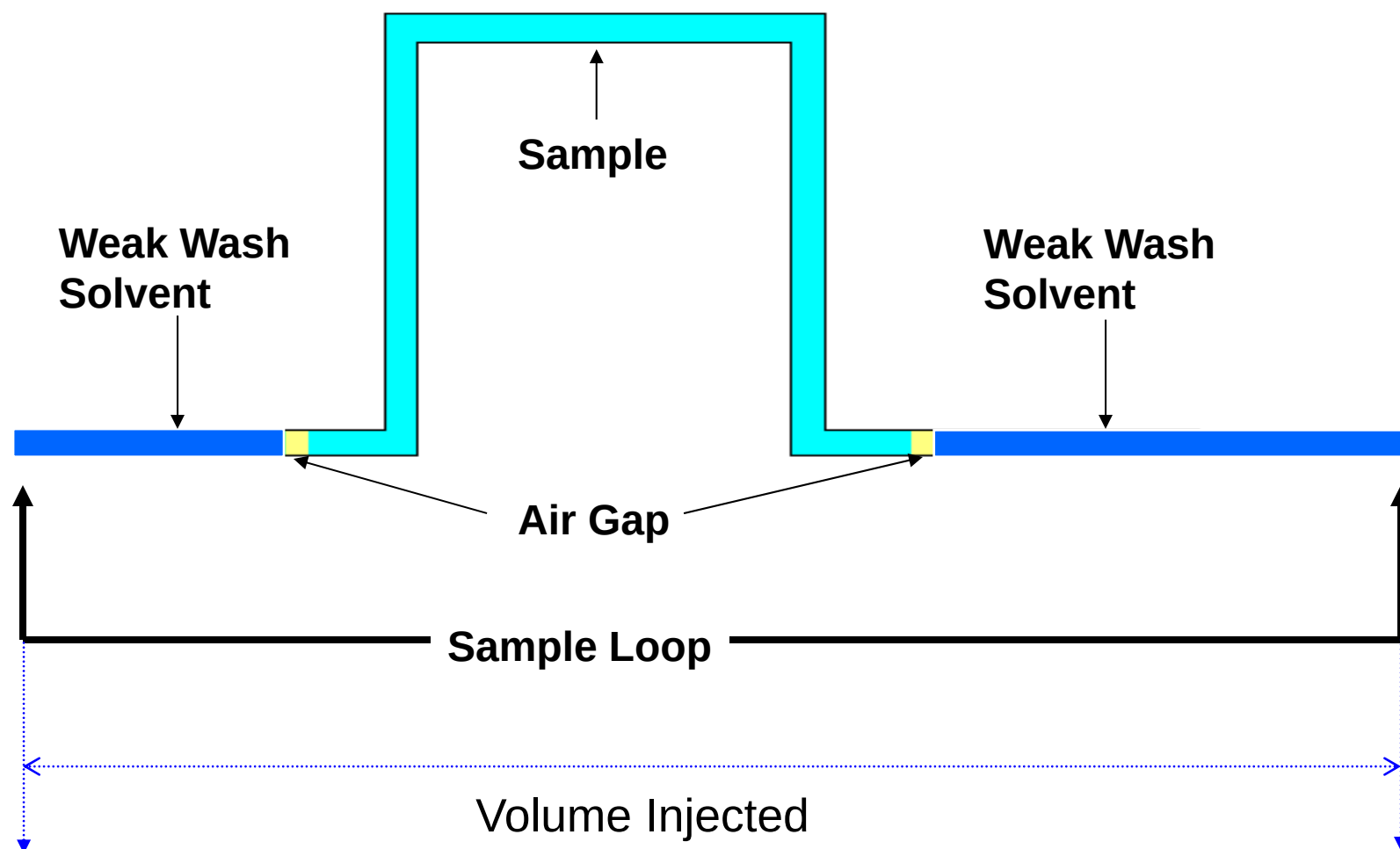


Partial Loop Injection Sequence- Step 3

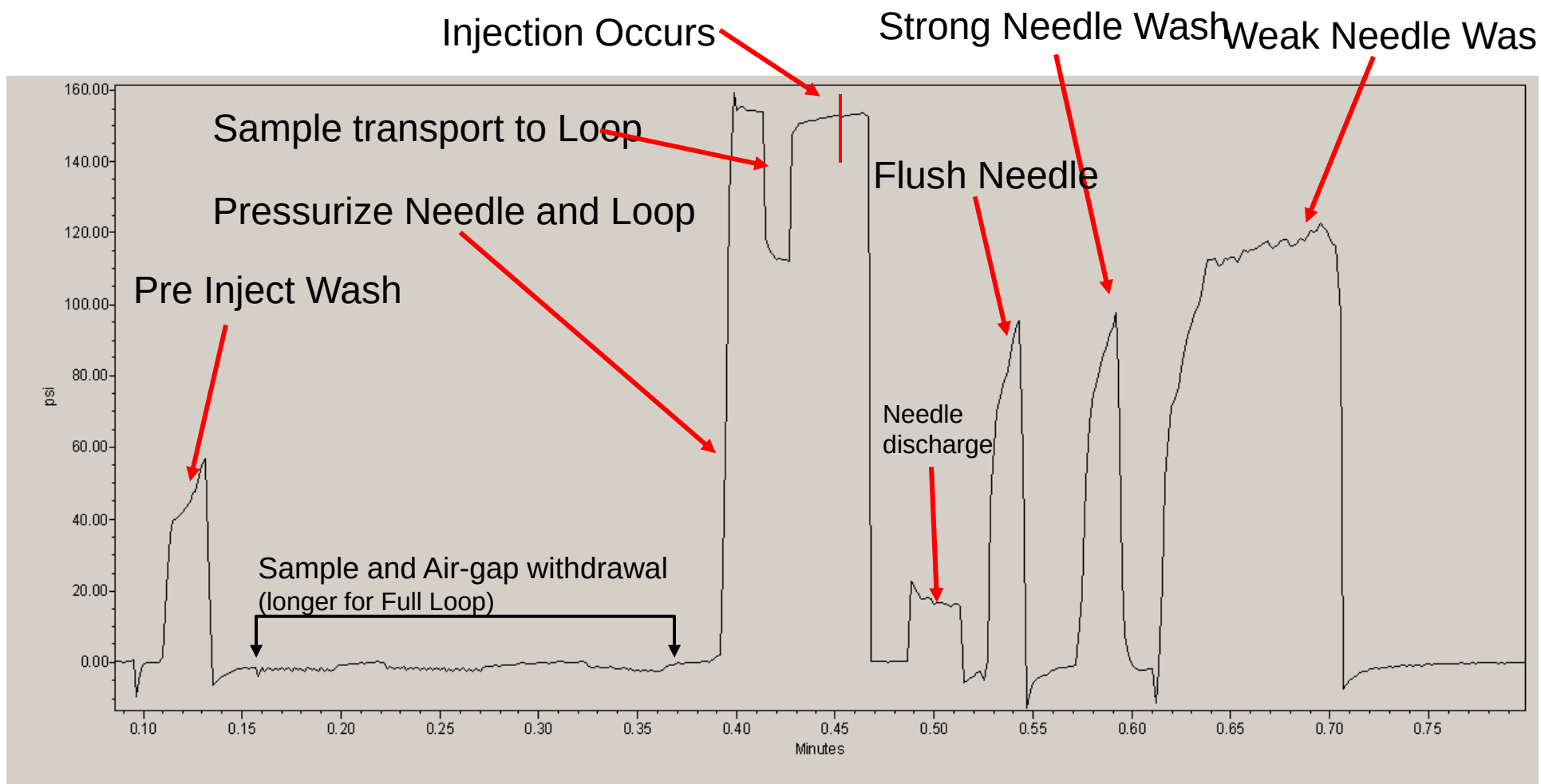


The injection sequence is then followed by weak and strong needle wash purge of the entire sample manager fluidics

Injection Loop Contents- Pressure Assist



Sample Pressure Trace- Full and Partial Loop with Pressure Assist



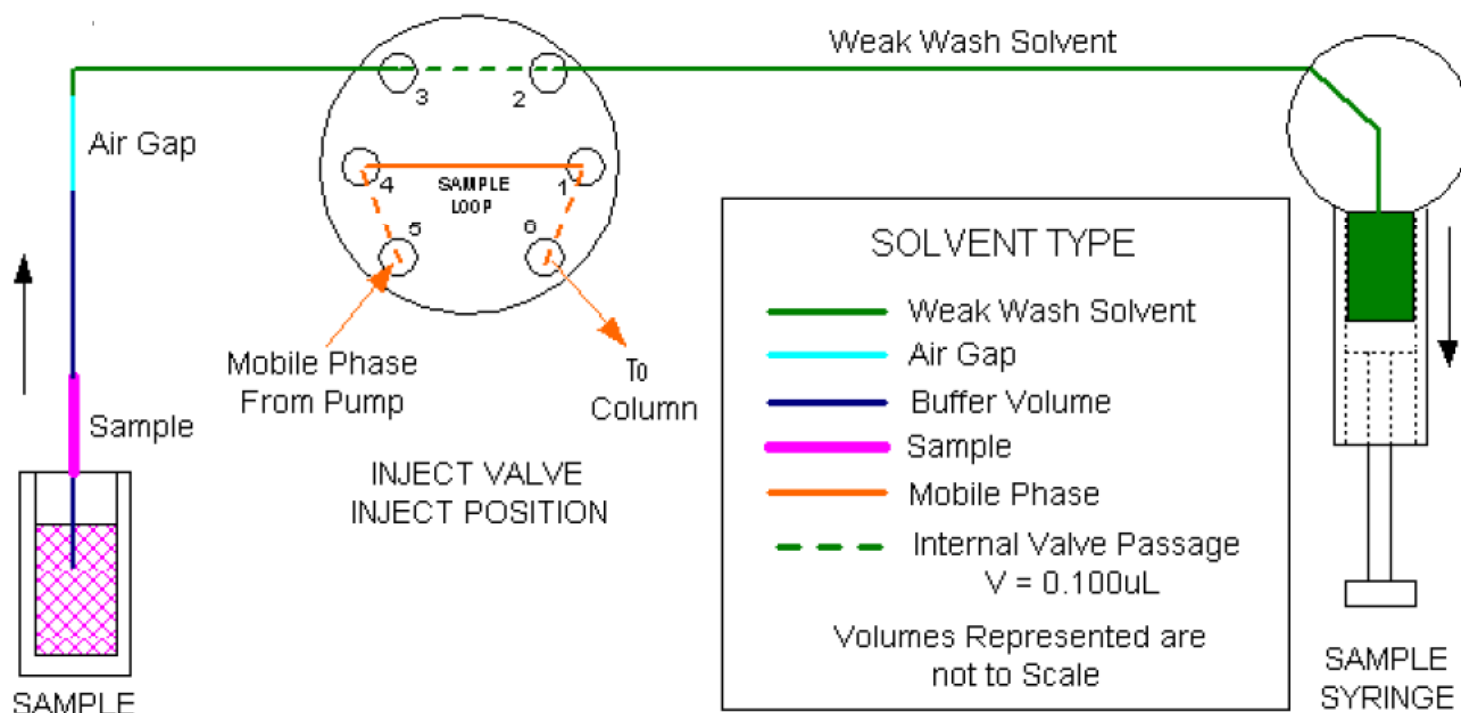
Partial Loop Using Needle Overfill Mode



PLUNO

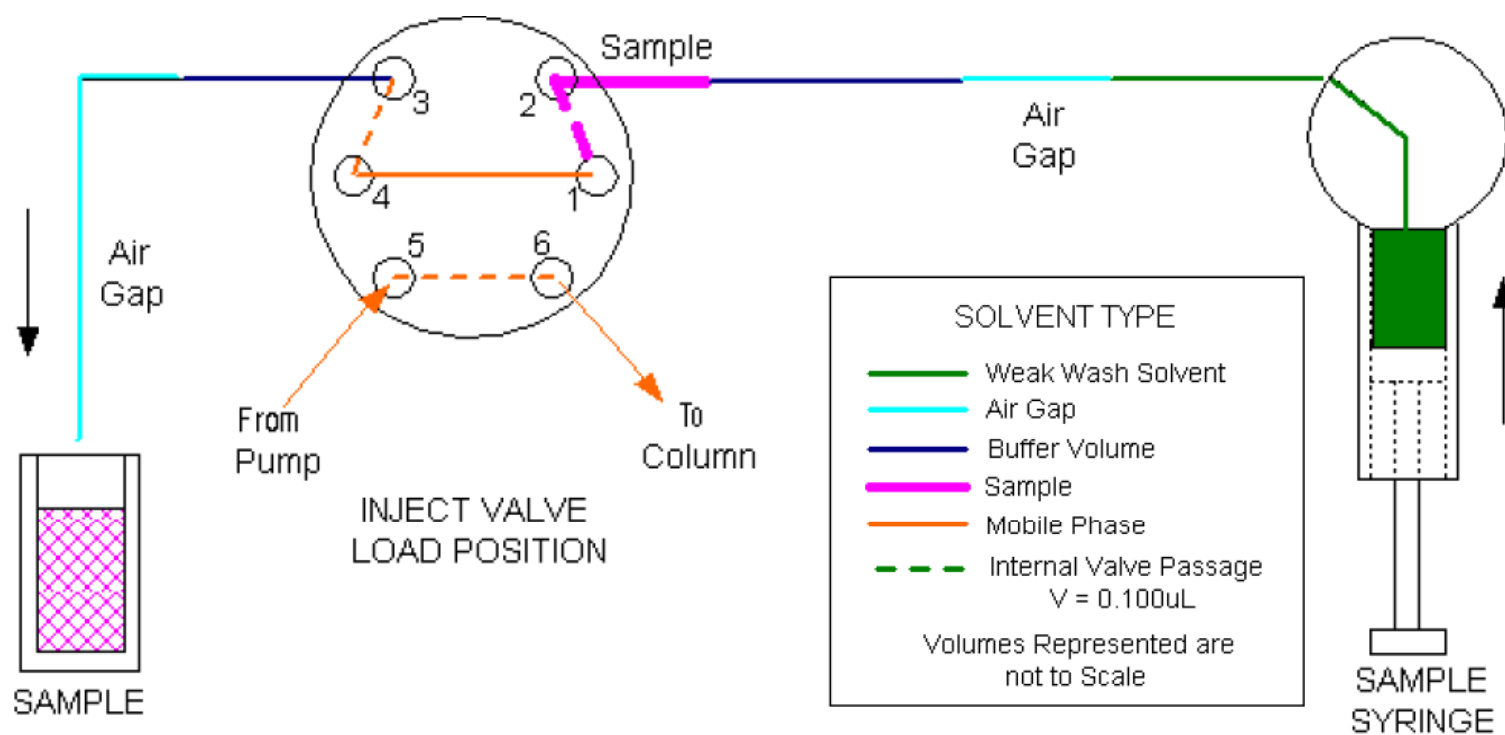


- Step 1
 - Sample Loop is in the Inject position
 - Sample Syringe draws an air gap and the sample volume plus 15ul
 - We call this the Buffer volume, 14uL pre and 1ul post volume



- [illegible]

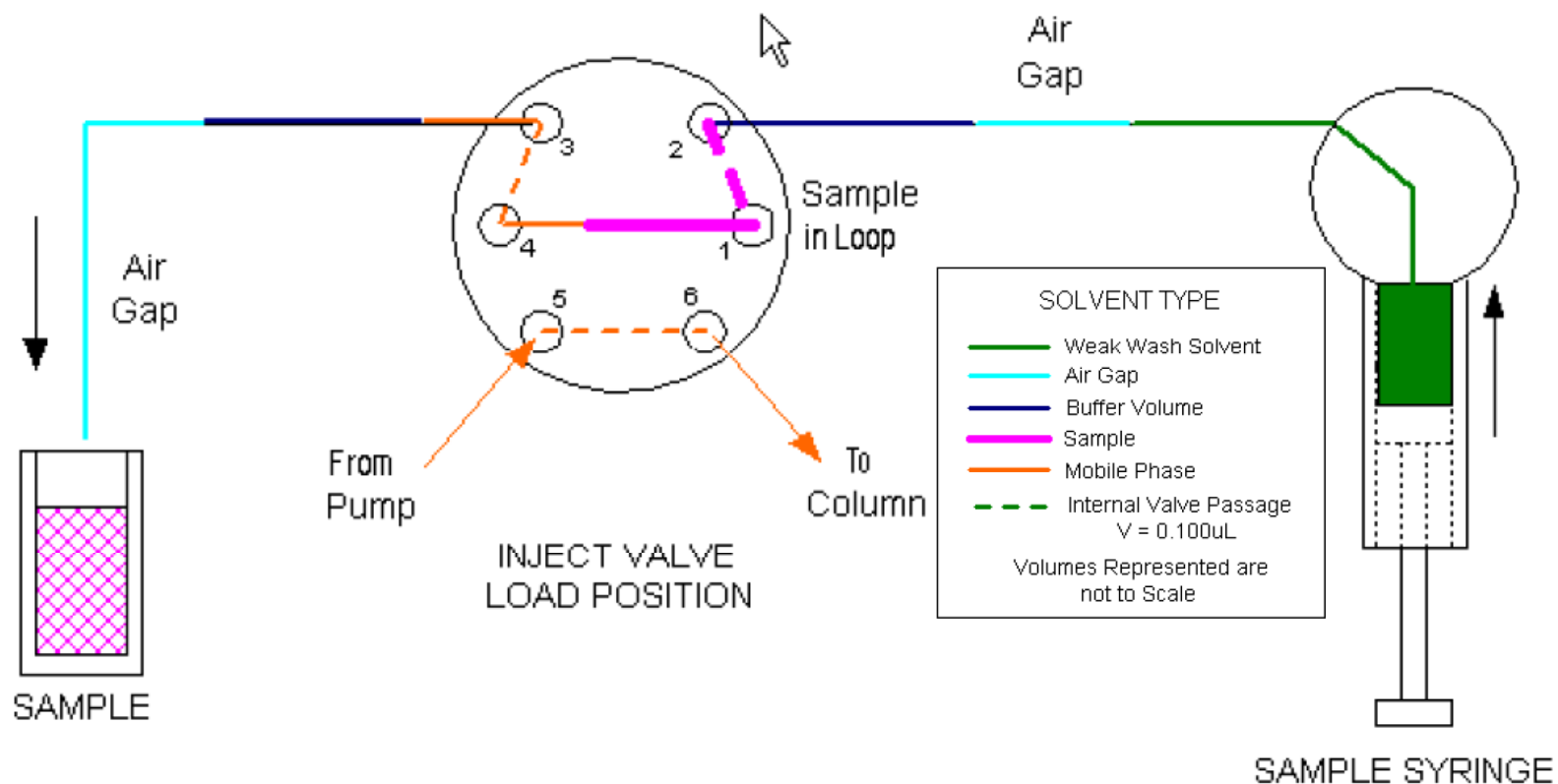
- Step 3:
 - The valve actuates bringing the sample loop into the Load position
 - The sample syringe pushed the sample back towards the needle



PLUNO



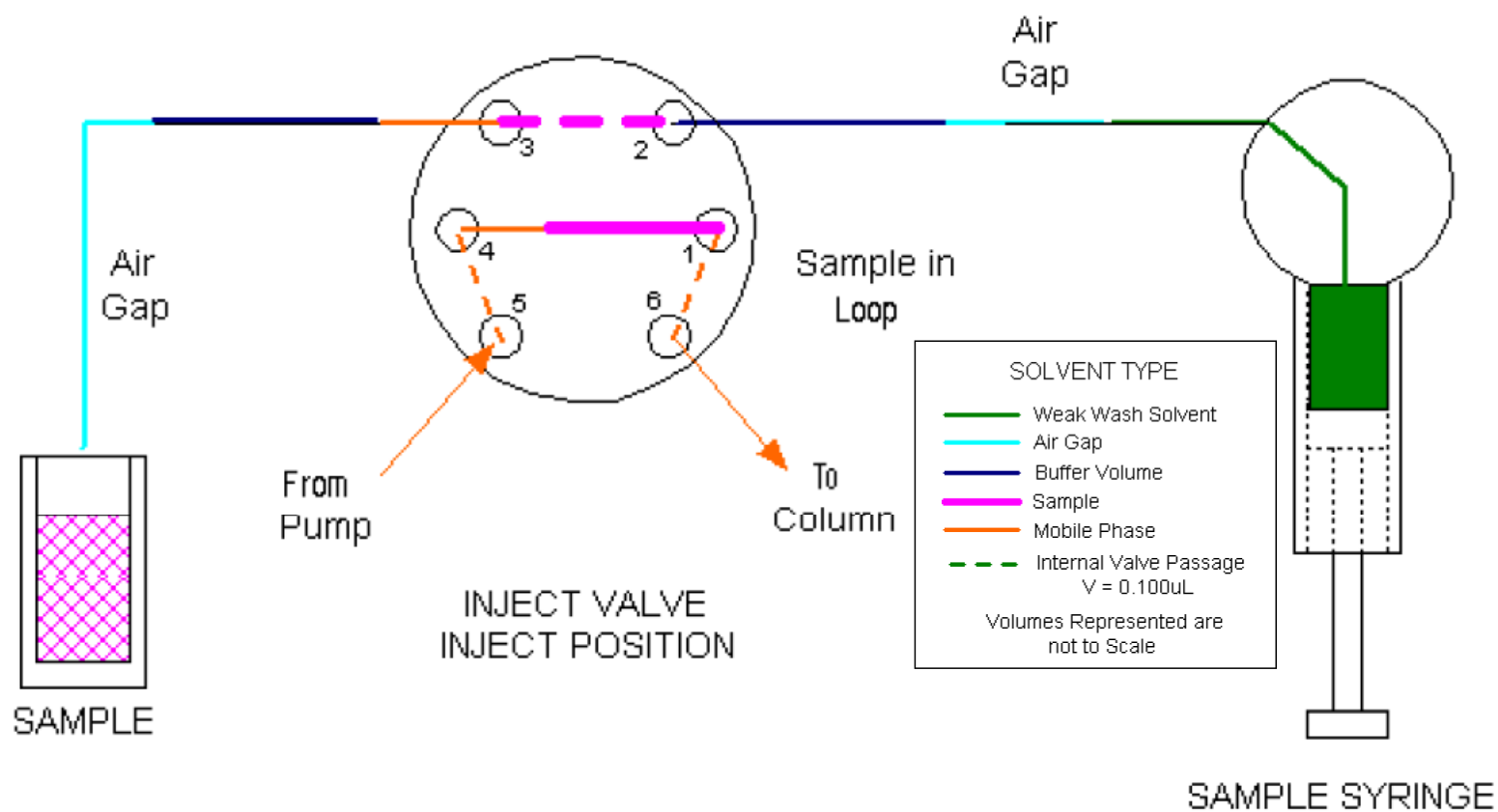
- Step 4:
 - Only the volume selected in the method is back loaded onto the Sample Loop



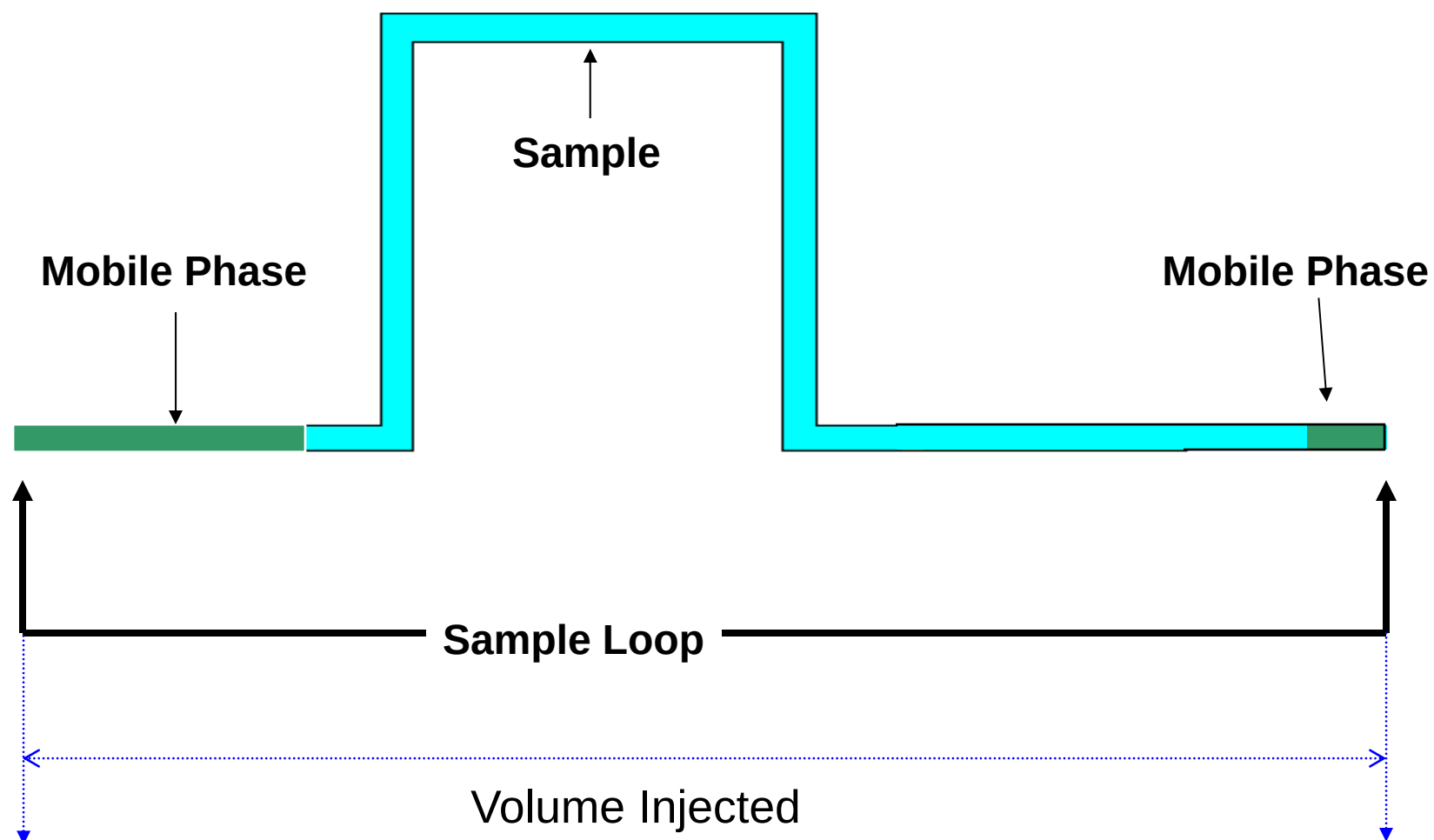
PLUNO



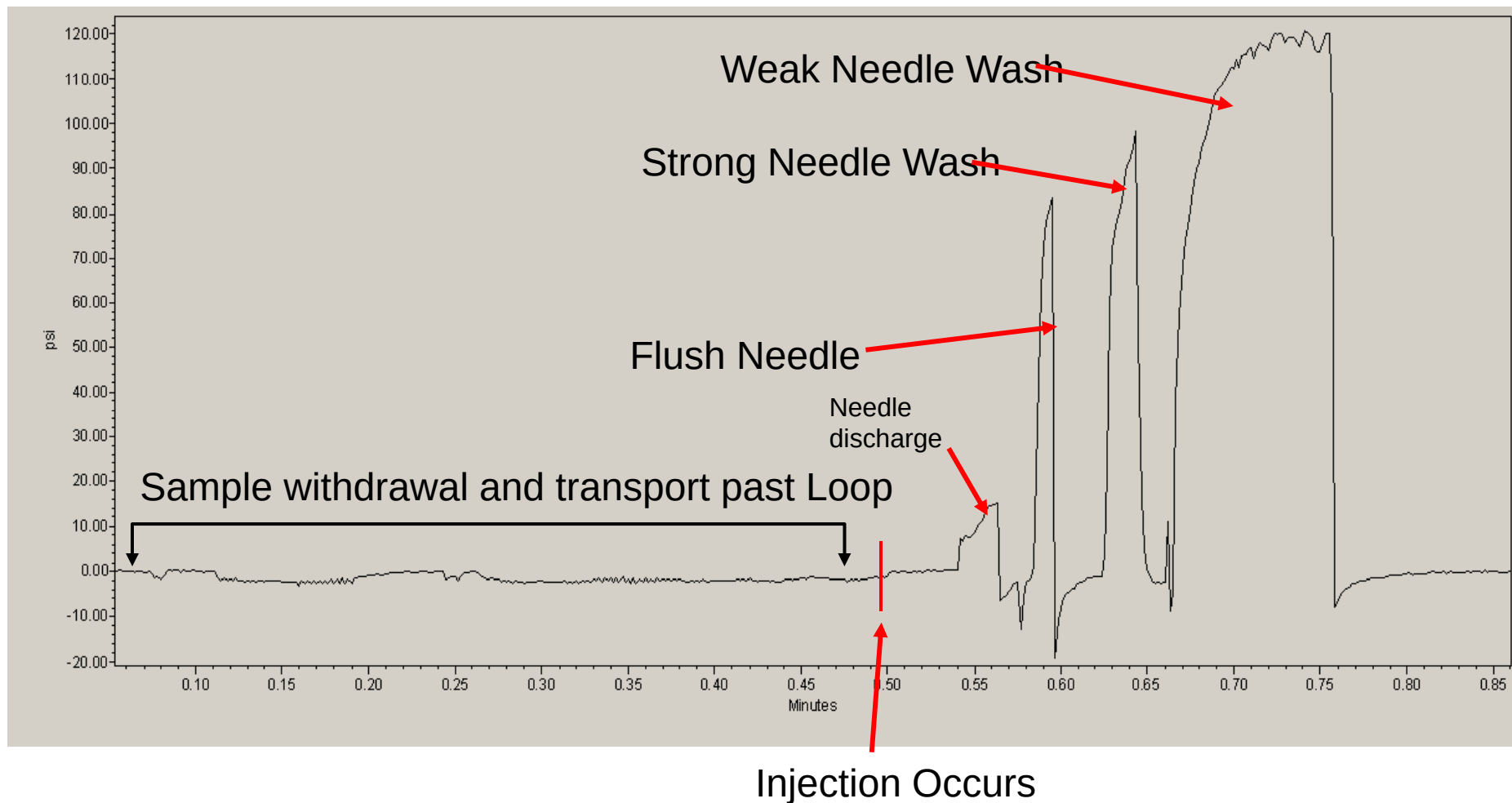
- Step 5:
 - The Inject Valve actuates bringing the sample loop into the “Inject” position
 - The sample is loaded on to the column



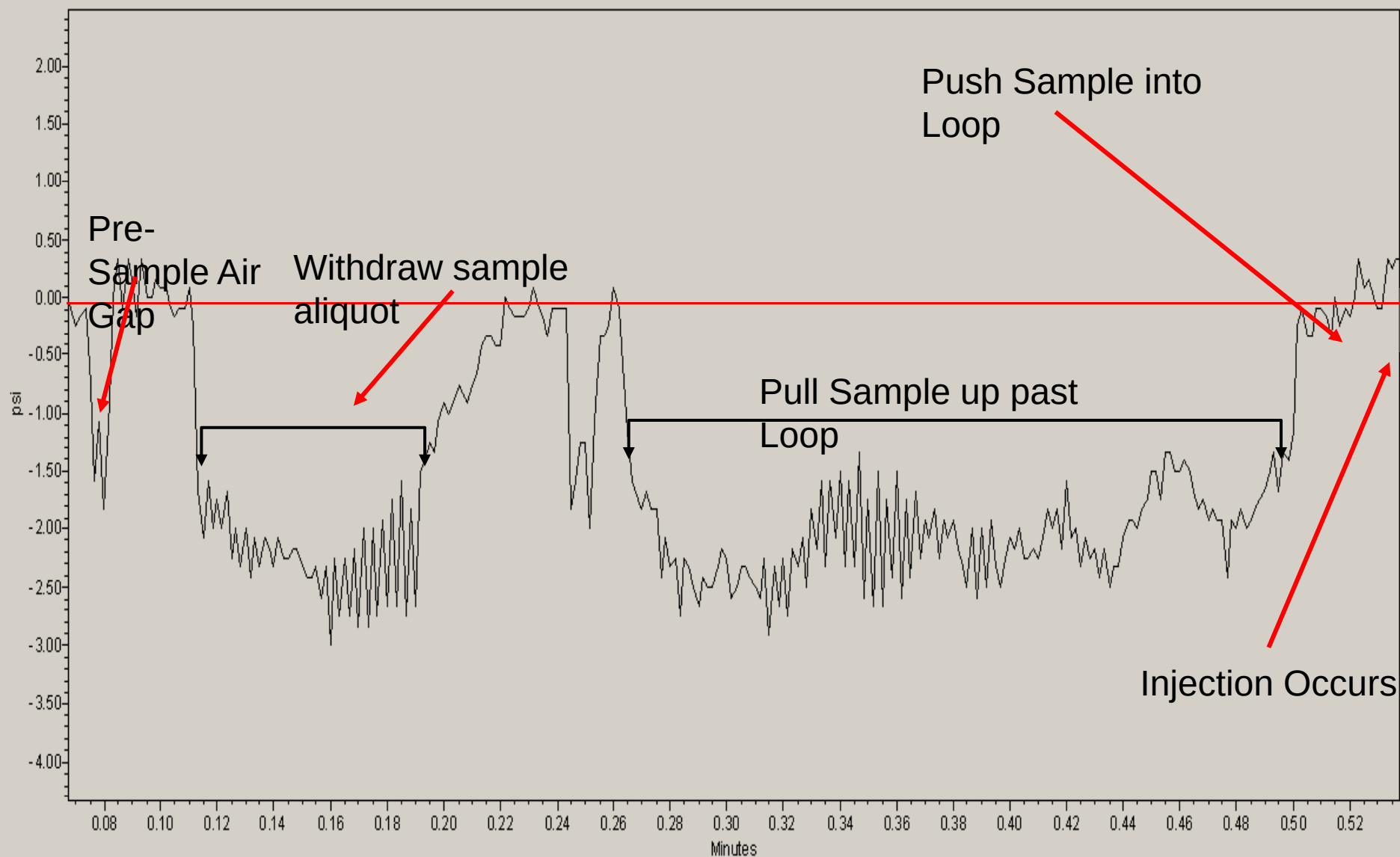
Injection Loop Contents- PLUNO



Sample Pressure Trace- PLUNO



Sample Pressure Trace- PLUNO



Partial Loop Using Needle Overfill Mode



- The best general purpose mode for partial loop injection
- Provides optimum performance when injection volumes are kept within a range of 20 – 75% nominal loop volume
- It provides the best partial loop accuracy, precision and linearity for a wide range of samples including
 - strong and weak acids and bases
 - hydrophilic and hydrophobic compounds.

Sample Manager - *Injection Modes*



Loop volume	Partial loop with needle overfill mode Recommended sample injection volume range (µL)	Partial loop (pressure assist) mode Recommended sample injection volume range (µL)	Full loop mode Recommended sample injection volume (µL)	Automatic overfill factor
1 µL	0.2 to 0.75	Not recommended	1	5.8
2 µL	0.4 to 1.5	Not recommended	2	5.6
5 µL	1.0 to 3.8	Not recommended	5	5.0
10 µL	2.0 to 7.5	2.0 to 5.0	10	4
20 µL	4.0 to 15.0	4.0 to 10.0	20	3
50 µL	10.0 to 37.5	10.0 to 25.0	50	2
100 µL	20.0 to 75.0	20.0 to 50.0	100	2
250 µL	40 to 150	40 to 125	250	1.5

Criteria for partial loop modes:

R^2 : > 0.999

Area %RSD: < 1%

Criteria for full loop mode:

R^2 : > 0.999

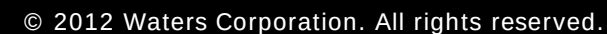
Area %RSD: < 0.3%

Limitation: Using 100-µL sample loops with 250-µL syringes, the injection linearity is restricted to 60% of the loop volume. The linearity for other combinations is 75% of the loop volume.

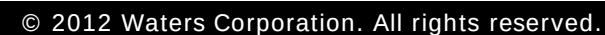
Needle-Wash System



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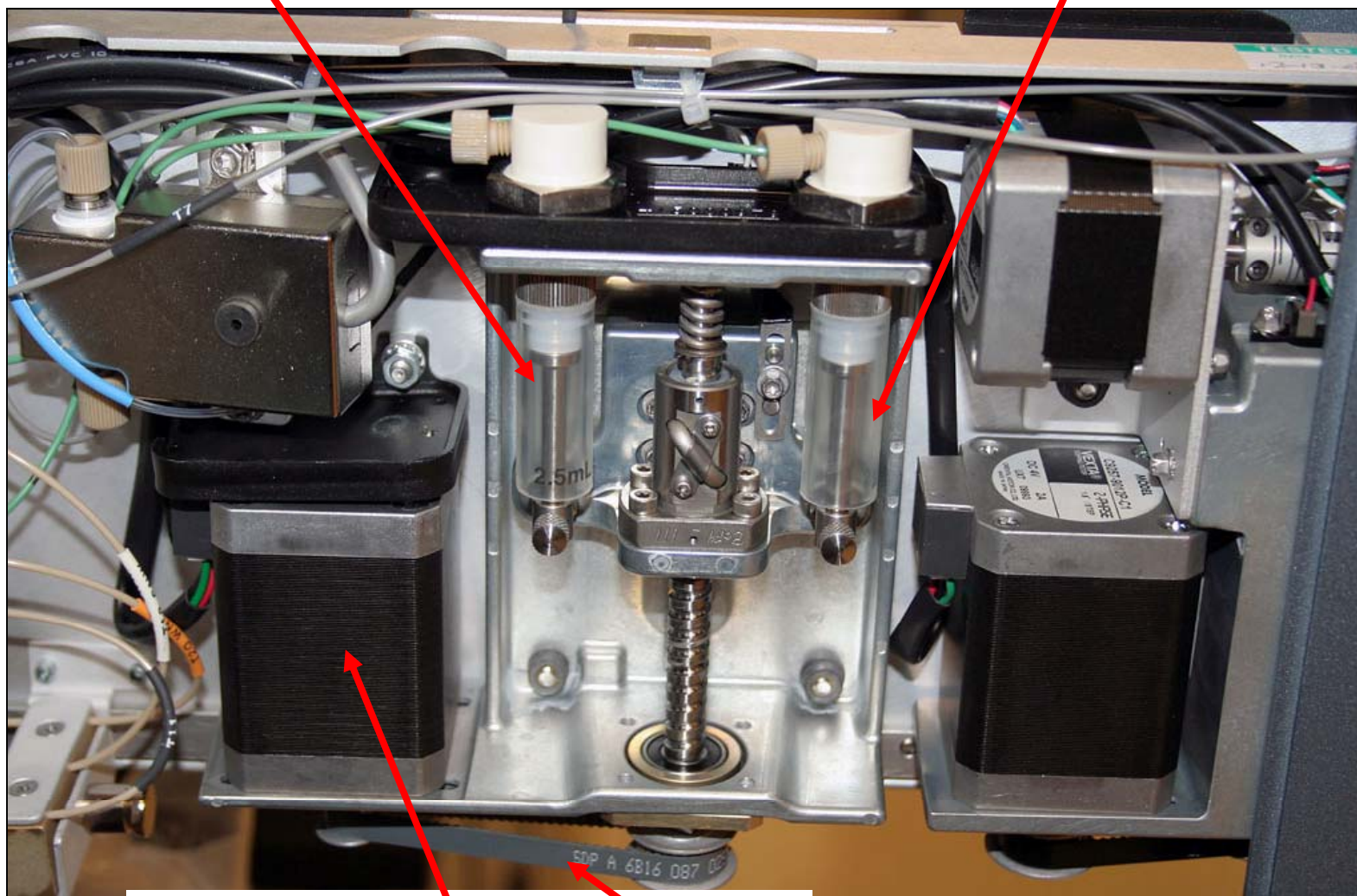
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Wash Syringes

Weak Wash Syringe

Strong Wash Syringe



Wash Syringe Drive Motor and Belt

Wash Solvent Considerations



Wash Solvent Considerations



- As a general principle, strong and weak solvents should include the same organic species
 - This may not always be practicable, especially in the case of “sticky” samples. You may, however use a 100% organic strong wash solvent
- For best results, weak wash solvent should be equivalent to the following (excluding buffers):
 - mobile phase composition (for isocratic separations)
 - initial gradient condition (for gradient separations)
 - If you dilute the samples, match the weak wash solvent to the sample diluent
- Do not use salt buffers in wash solvents

Wash Solvent Considerations



- Strong solvent should be no stronger than the concentration needed to reduce carryover to an acceptable level
- Strong wash solvent should not contact the sample
- Wash volume ratio (weak to strong)
 - Should be about 3:1, weak wash to strong
 - Sufficient to ensure the weak wash flushes the strong from the needle and sample loop
- For more details on solvents, see the section titled “Selecting weak wash and strong wash solvents” in the ACQUITY Operators Guide

Characterizing the Needle and Loop Volume

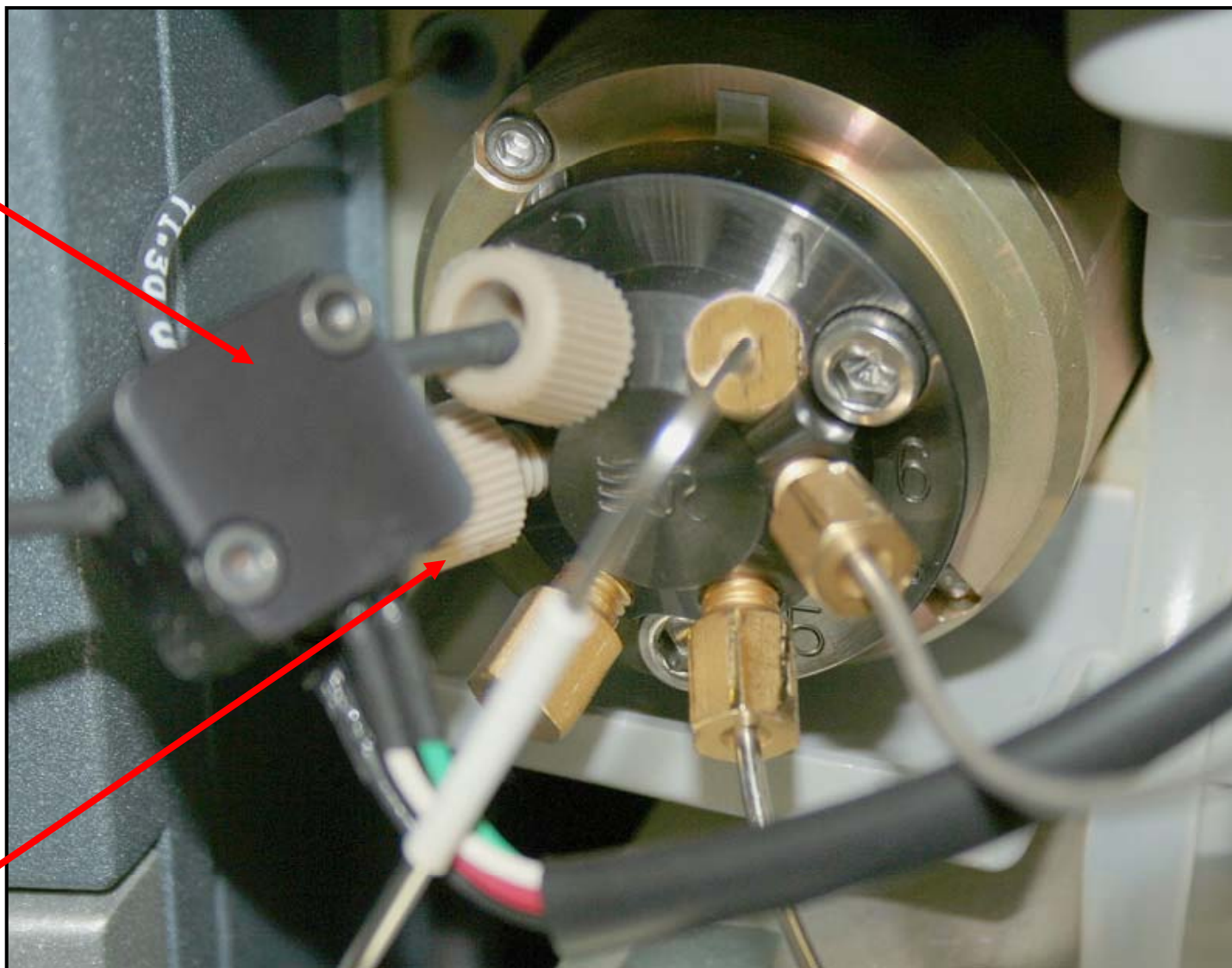


Volume Detection Device (Bubble Sensor)



VDD
(Bubble
Sensor)

To
Needle



Volume Configuration Menu

A screenshot of a software dialog box titled "Volume Configuration". It contains three configuration options, each with a text label, a numeric input field, a unit label, and a dropdown arrow. The first option is "Loop size:" with a value of "10.0" and unit "µL". The second is "Needle size:" with a value of "30" and unit "µL". The third is "Sample syringe size:" with a value of "100.0 µL". At the bottom of the dialog are two buttons: "OK" and "Cancel".

Parameter	Value	Unit
Loop size:	10.0	µL
Needle size:	30	µL
Sample syringe size:	100.0 µL	

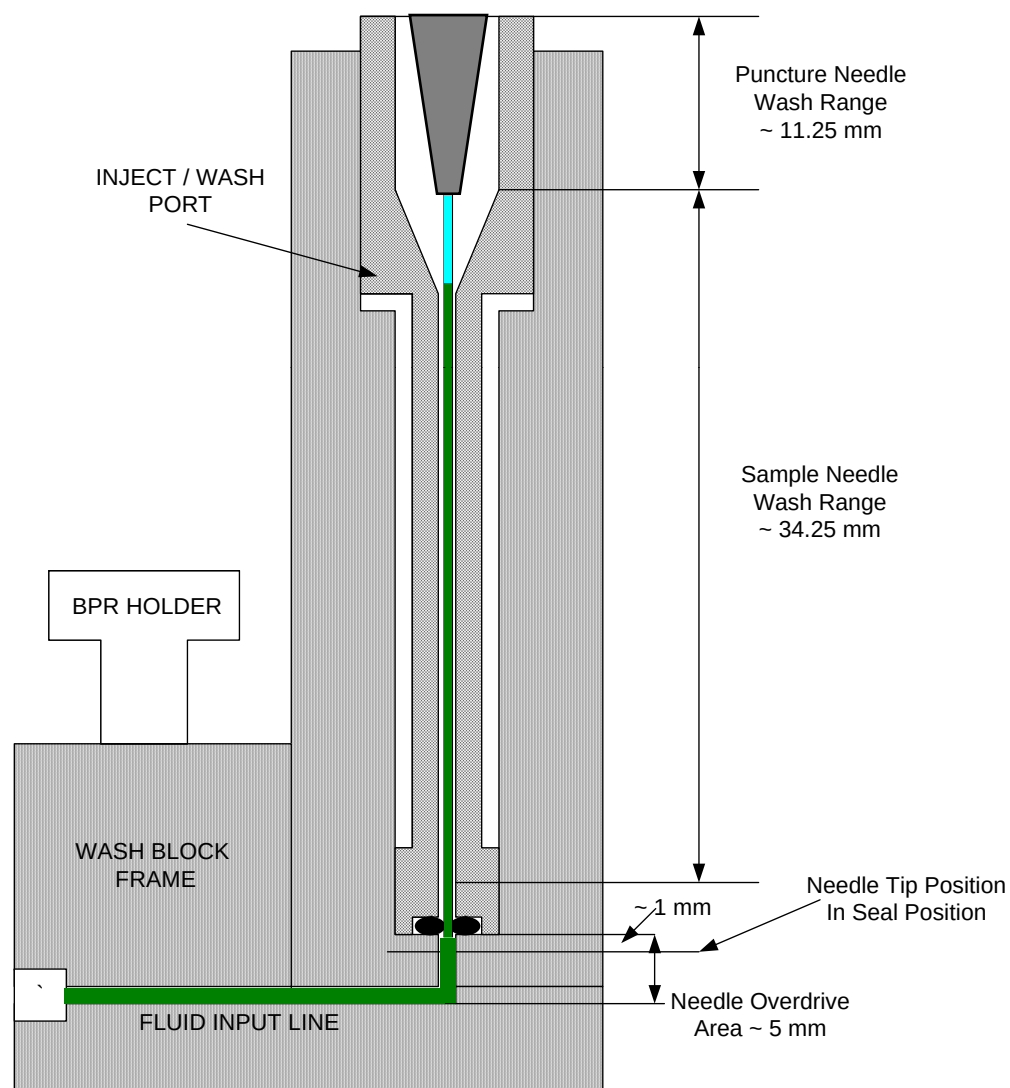
Before running the Characterize Needle and Loop Volume function, make sure the proper volumes are configured in the Volumes Configuration menu.

The above values are standard for the currently shipping ACQUITY system.

Wash Block- Pressure Assist Function

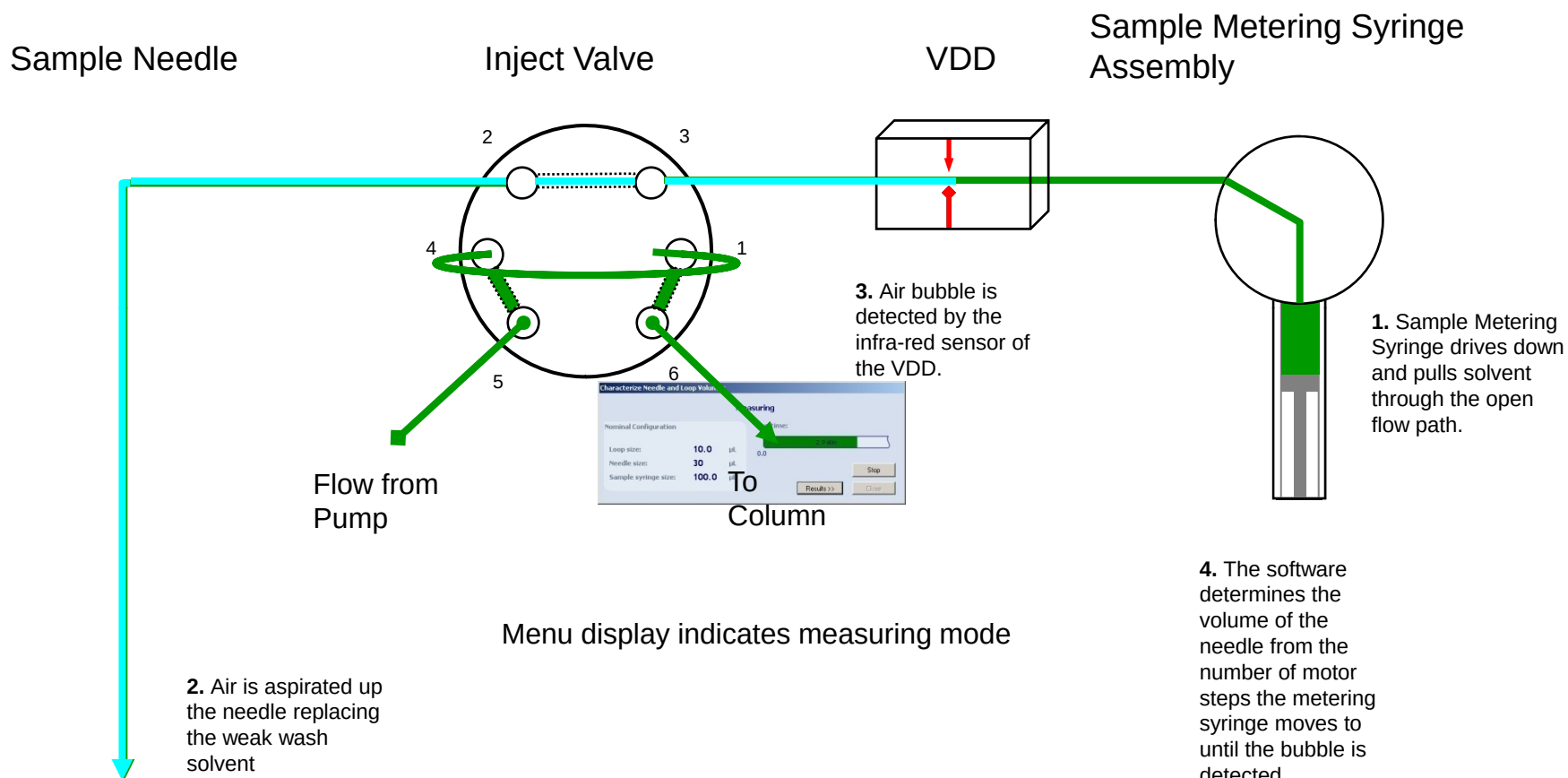


Wash / Inject Block Internal Detail

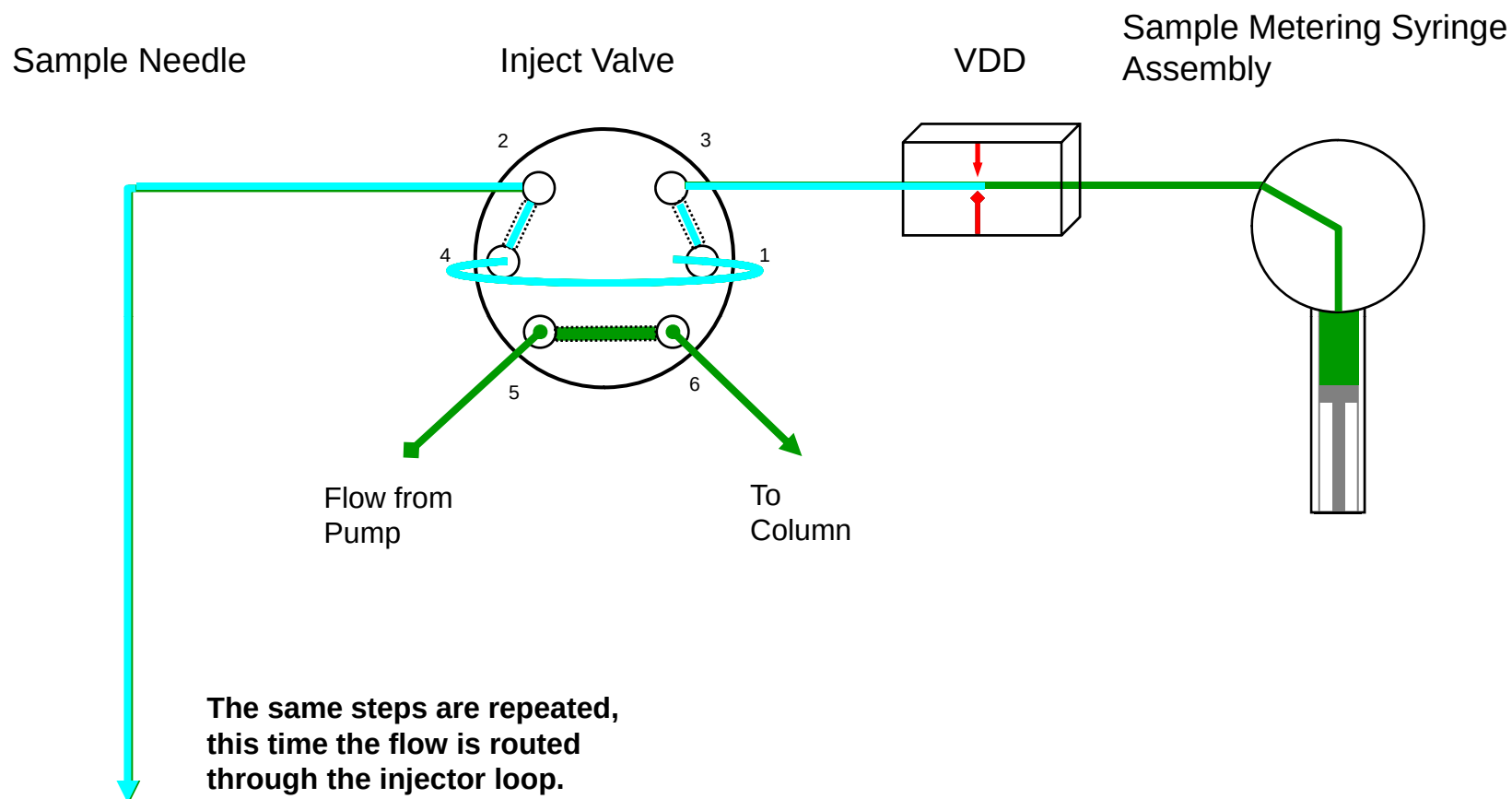


WASH / INJECT BLOCK

Characterize Needle Volume Function



Characterize Needle and Loop Volume Function

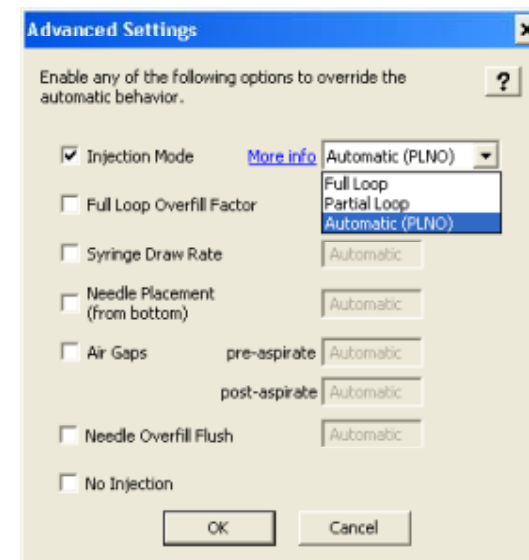


The results are calculated and displayed in the results menu

SM-FL: Injection Mode Recommendations

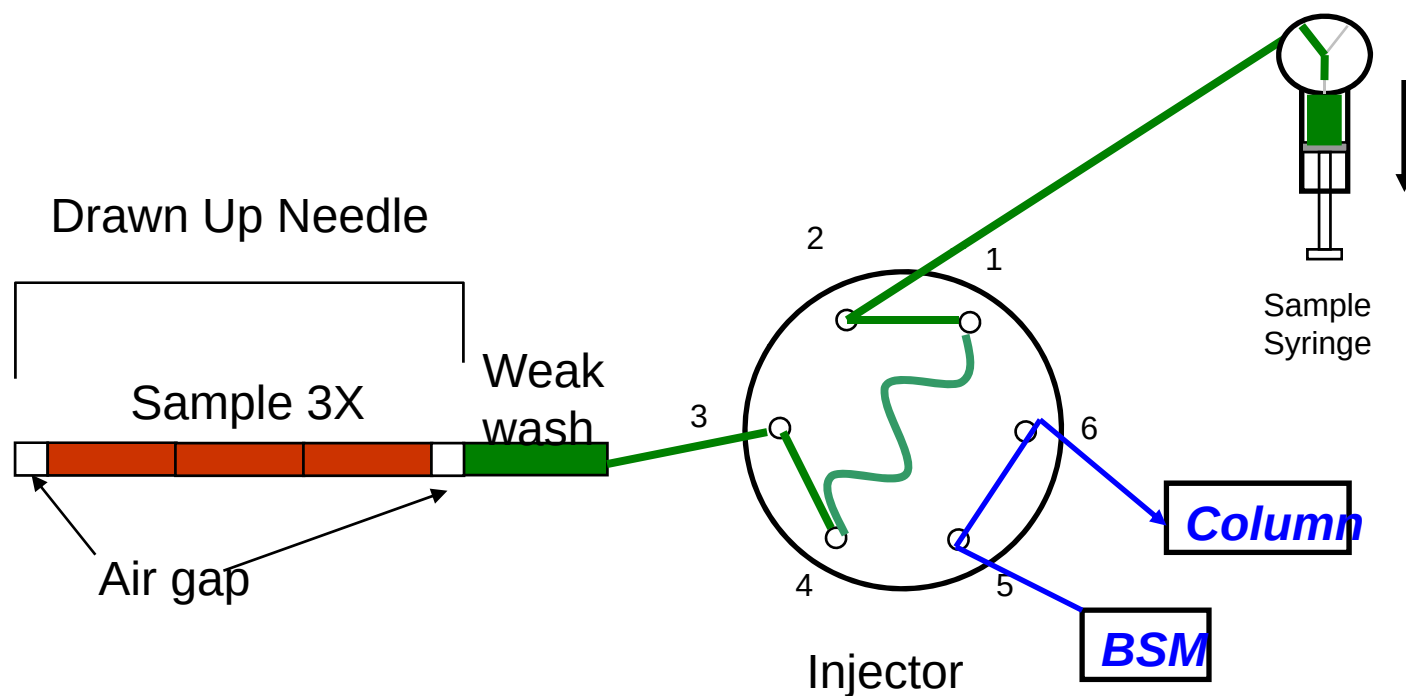


- Specified in the Advanced Settings
 - Full loop
 - Best precision
 - Requires lots of sample - 3x or more
 - Partial loop with pressure assist
 - Weak wash injected along with the sample
 - Inject up to 50% of loop volume
 - Recommended only for 20 and 50µL loops
 - Recommended for fast injection-to-injection cycles
 - No extra sample consumed
 - Partial loop needle overfill
 - Widest injection volume range: 10-75% of loop volume
 - More reproducible than partial loop with pressure assist
 - Requires minimum 3 µL more than the injection volume
 - Default will be less than 15µL, TBD



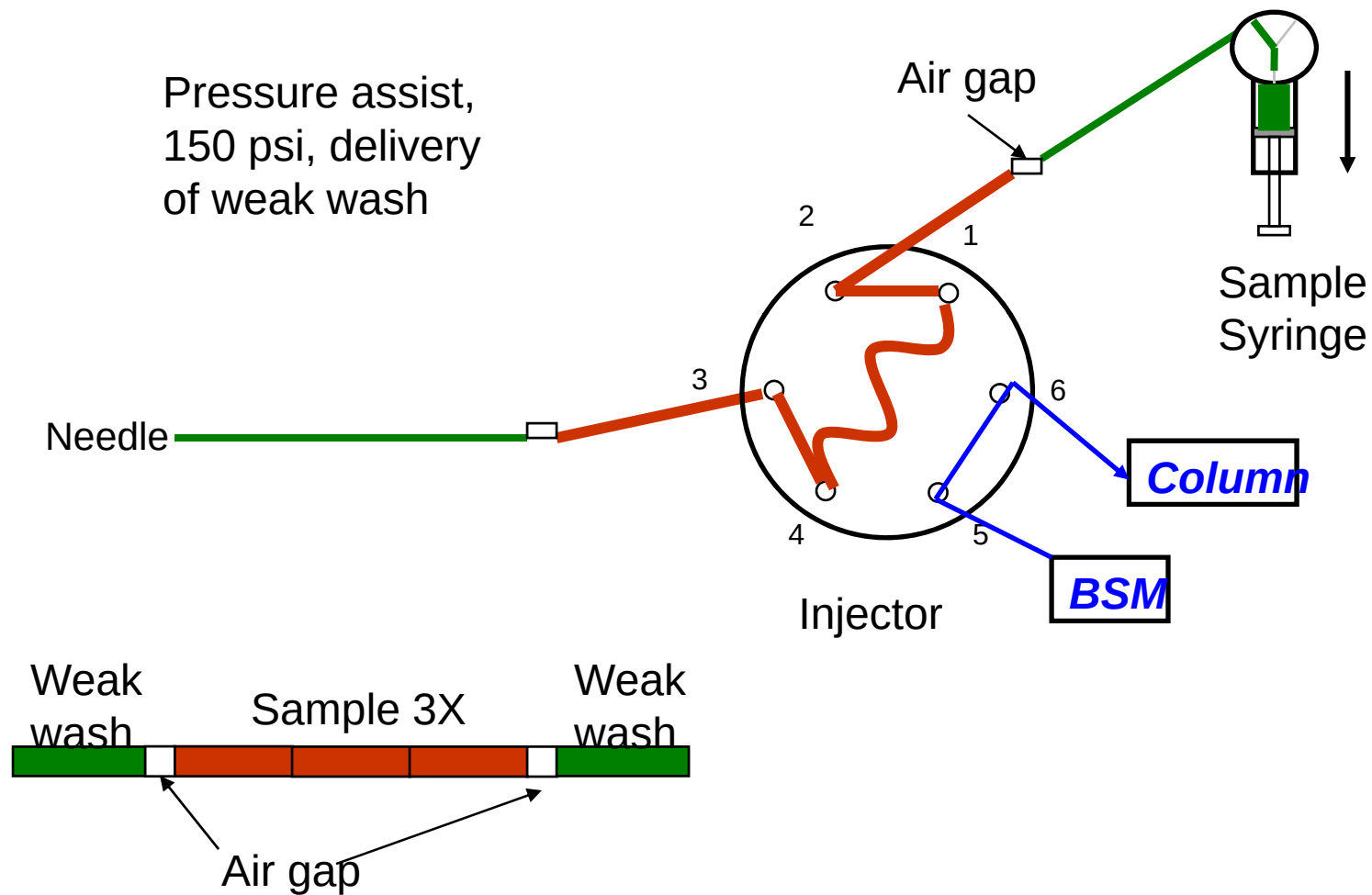
SM-FL: Full Loop - 1

Sample Drawn



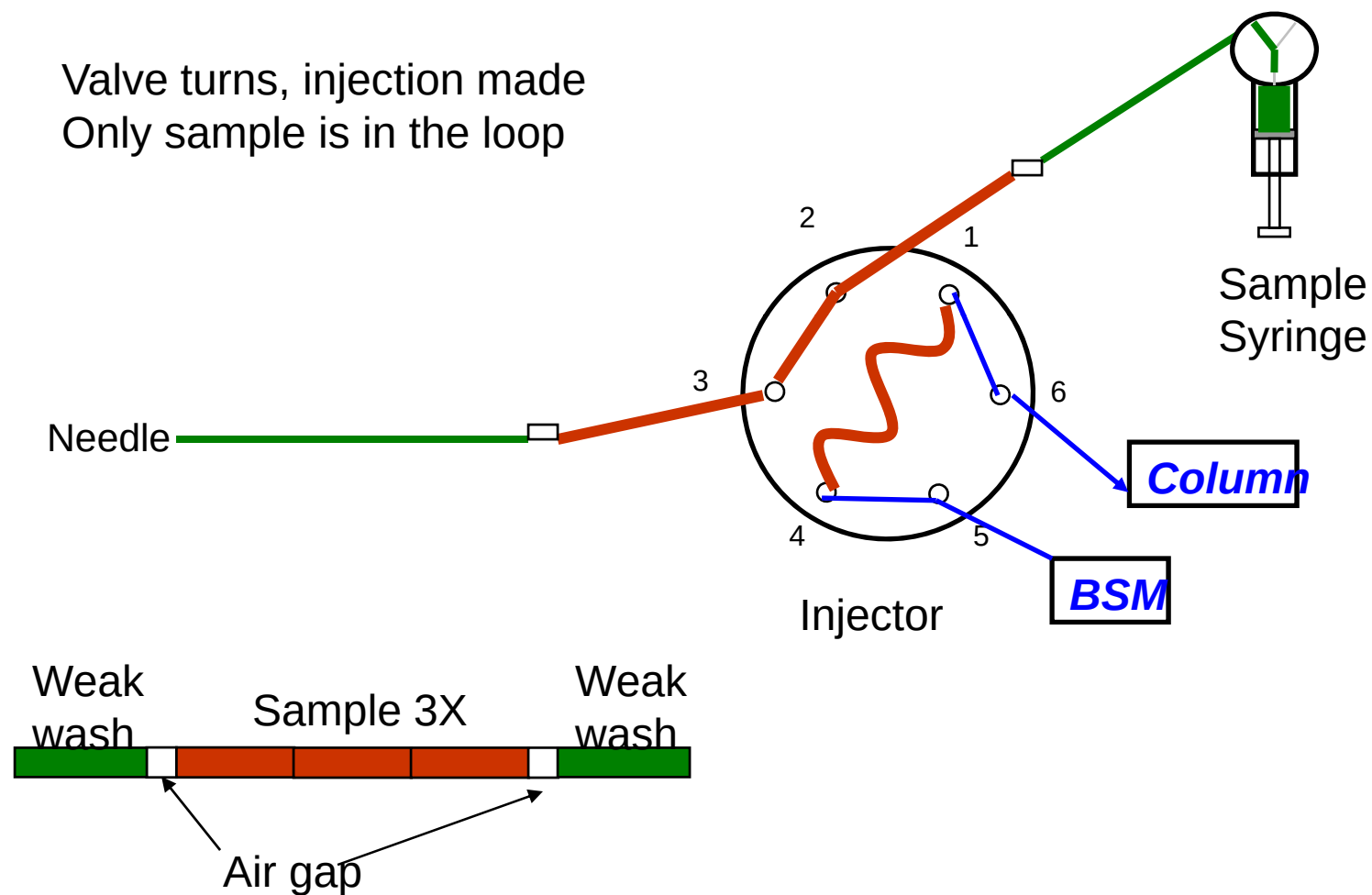
SM-FL: Full Loop - 2

Sample Loaded



SM-FL: Full Loop – 3

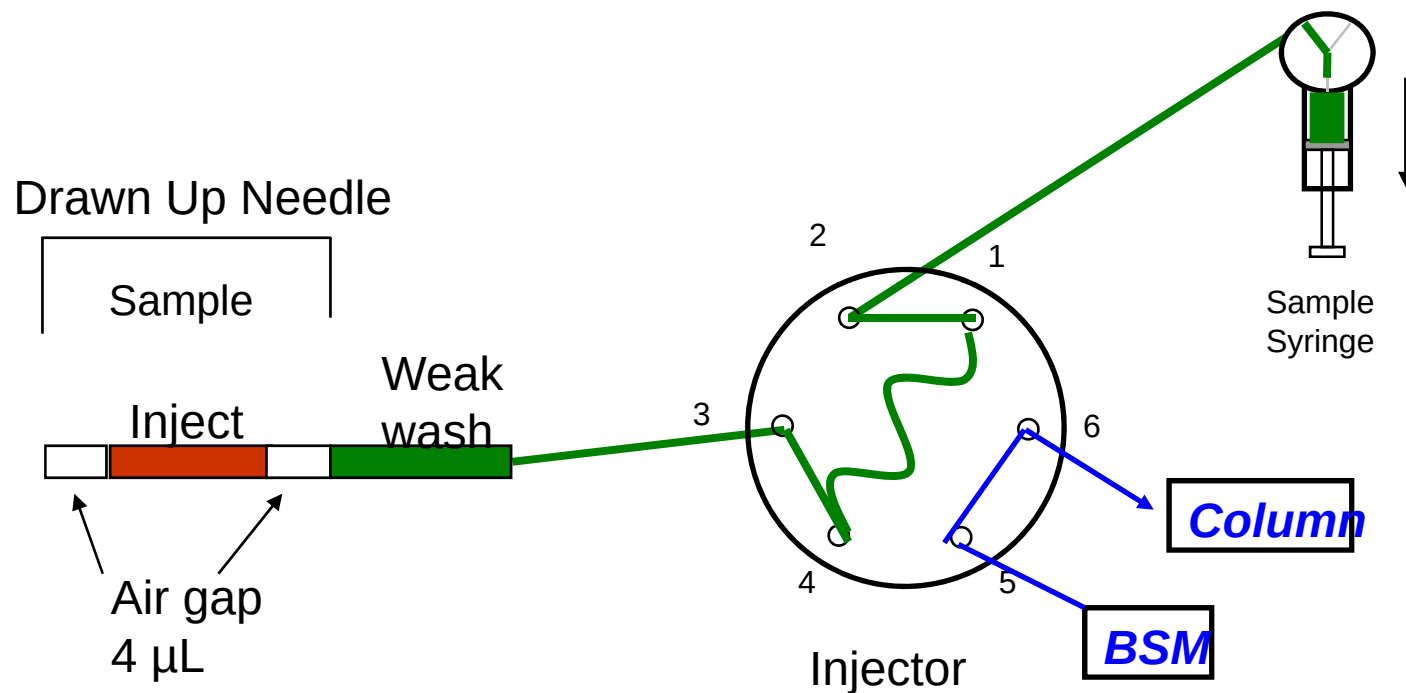
Sample Injected



SM-FL:

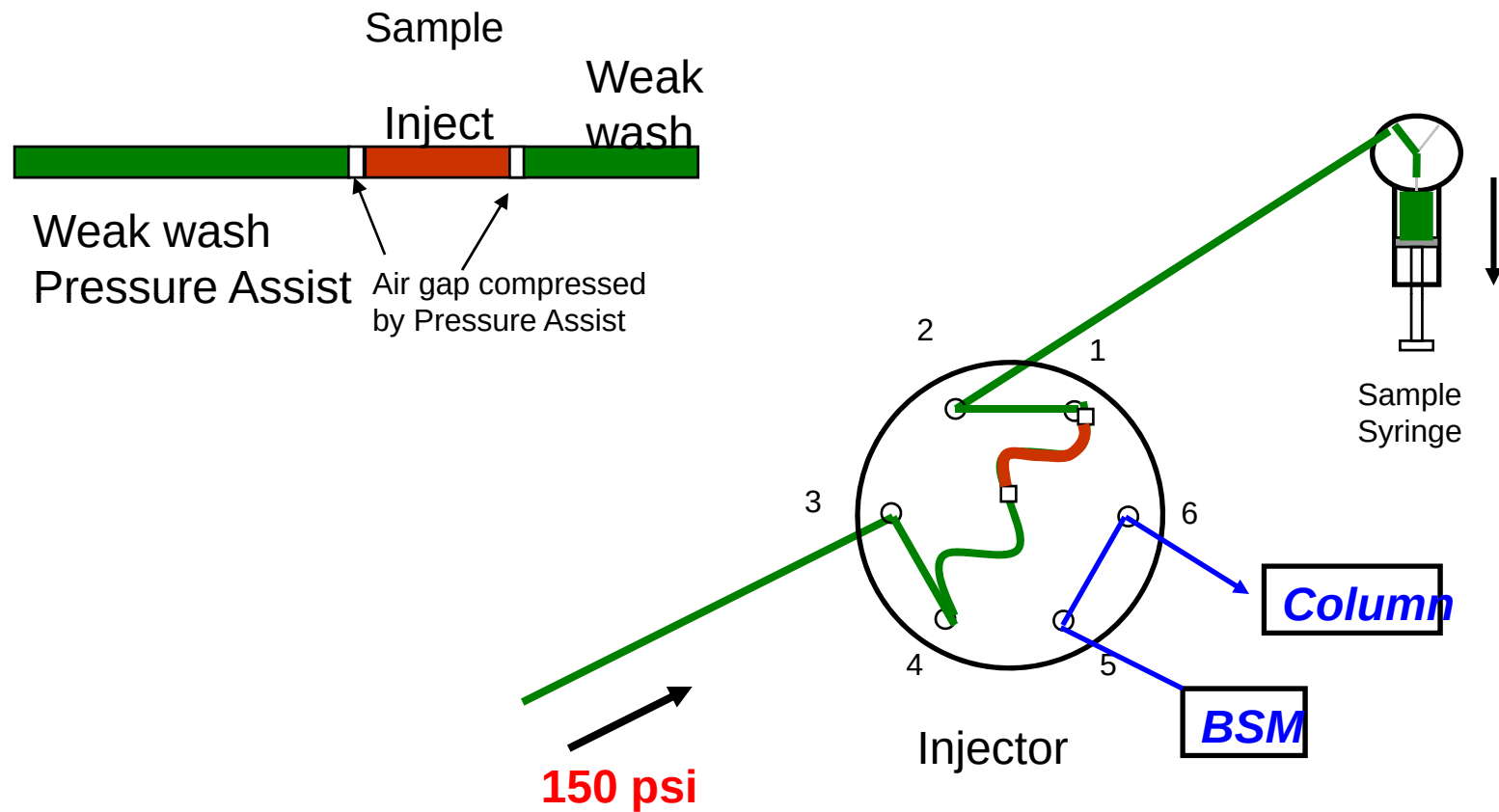
Partial Loop (Pressure Assist) - 1

Sample Drawn



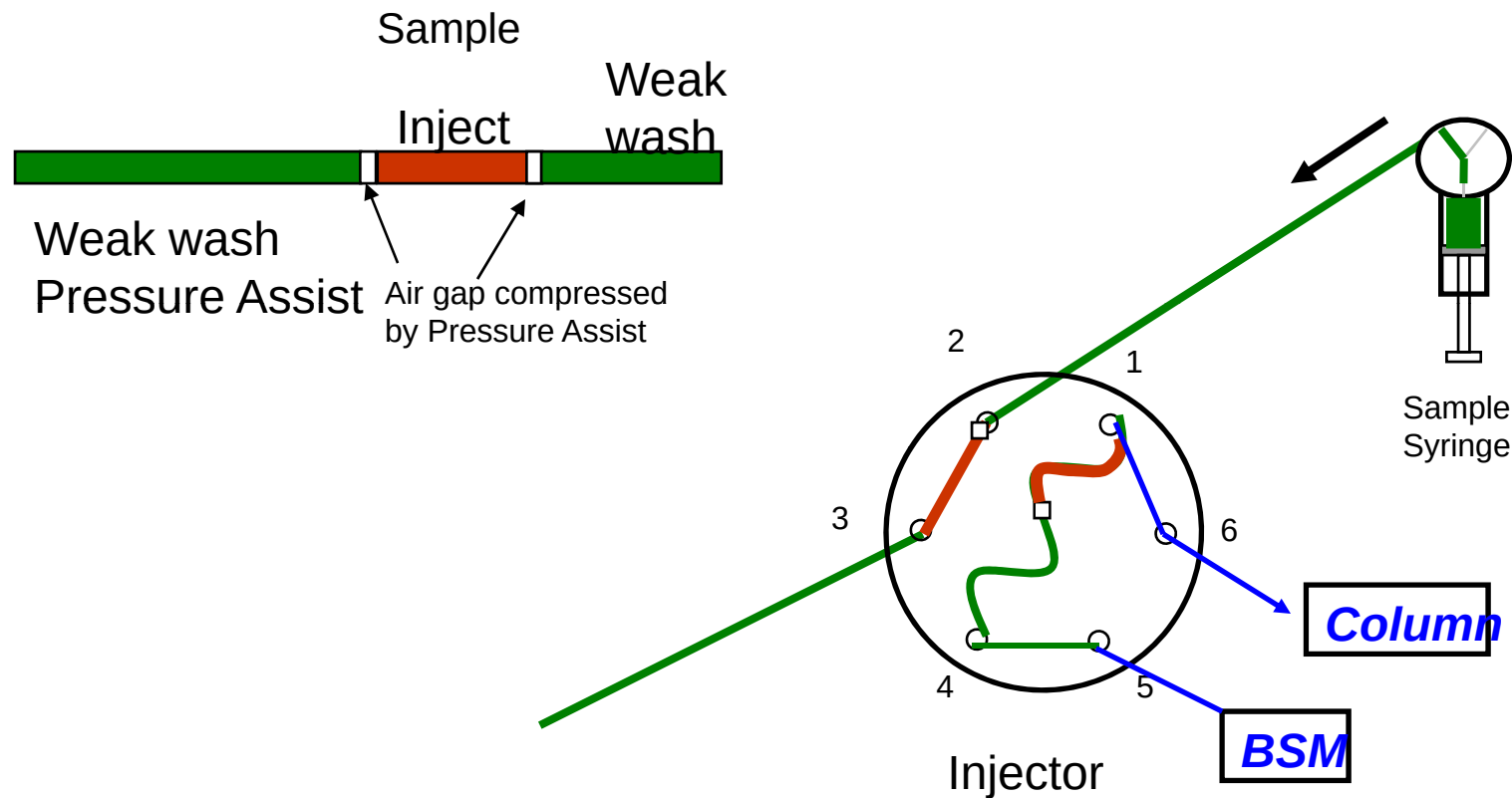
SM-FL: Partial Loop - 2

Sample Positioned



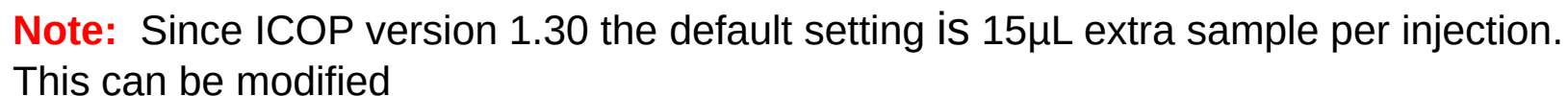
SM-FL: Partial Loop - 3

Sample Injected



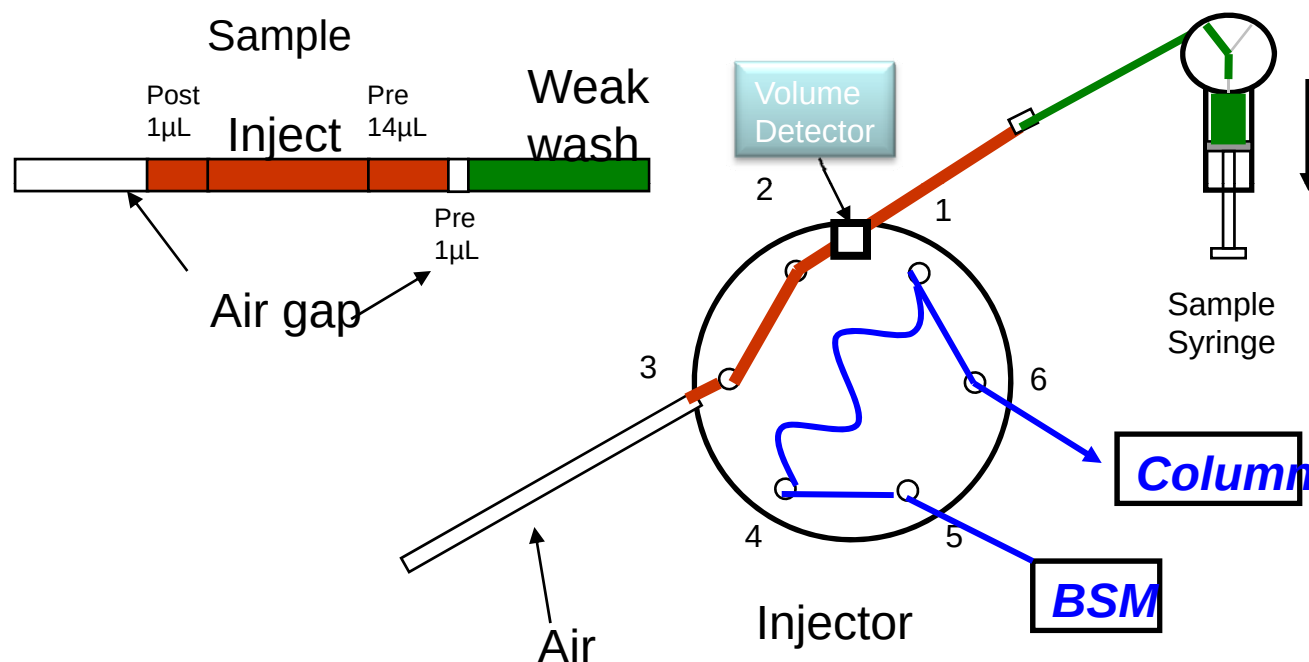
NOTE: Air gap injected

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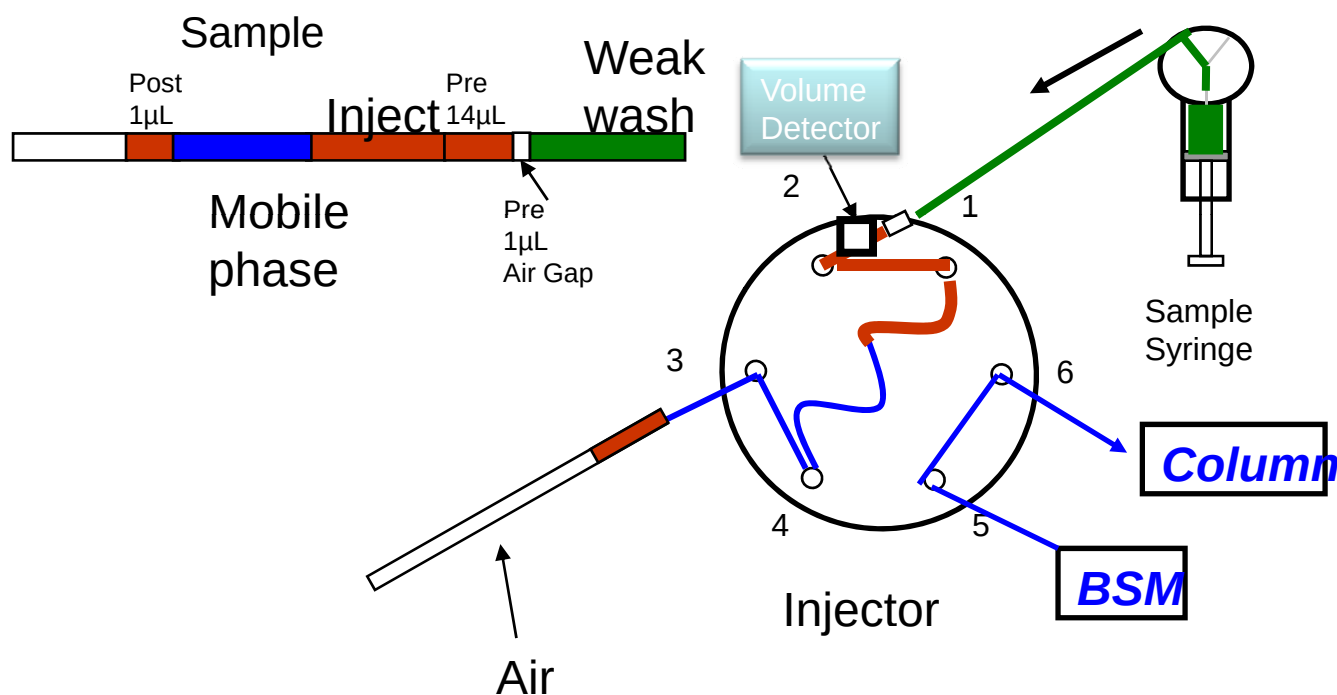
SM-FL: Partial Loop Needle Overfill - 2

Sample Positioned



- Sample positioned after the Injector Valve in the Volume Detector tube

SM-FL: Partial Loop Needle Overfill – 3 Sample Loaded

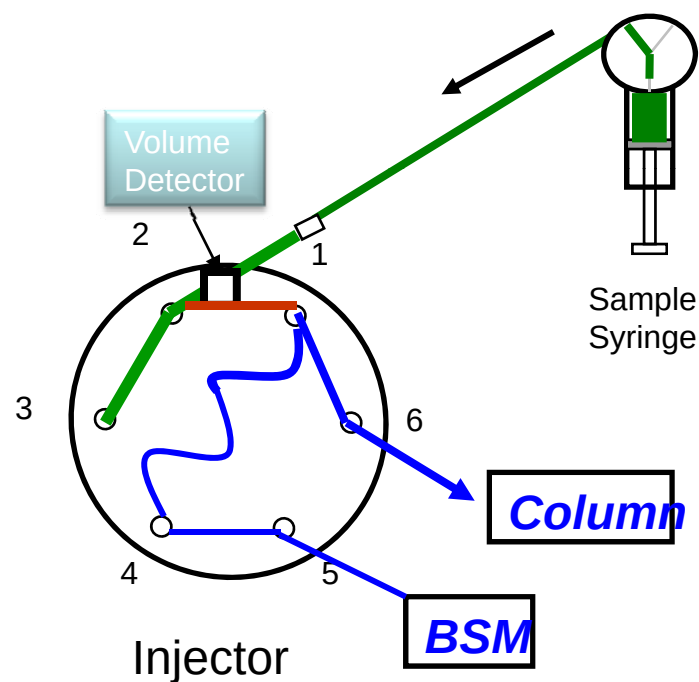


- Sample pushed into the Injector loop
- Mobile phase fills remainder of loop

SM-FL: 'Double-Click'



- After injection and cleaning of the valve and sampling mechanism there is a part of the valve fact that is not cleaned (Noted in RED)
- By actuating the valve during the run the material is pushed into the sample loop and then 'injected' during the end of the gradient



SM-FL: Cycle Inject Valve



Modify ACQUITY Sample Manager Instrument Method

Acquity Sample Manager UPLC*

Run Time: 6.50 min

General | Data | Events

Initial Switch States

1: No Change 3: No Change
2: No Change 4: No Change

Chart Out: Sample Pressure

☒ Run Events

	Time (min)	Event
1	2.00	Cycle Inject Valve
2		Switch 1
3		Switch 2
4		Switch 3
5		Switch 4
6		Sample Temperature
-		Column Temperature
-		Sample Temperature Off
-		Column Temperature Off
-		Cycle Inject Valve

Modify ACQUITY Sample Manager Instrument Method

Acquity Sample Manager UPLC*

Run Time: 6.50 min

General | Data | Events

Initial Switch States

1: No Change 3: No Change
2: No Change 4: No Change

Chart Out: Sample Pressure

☒ Run Events

	Time (min)	Event	Action
1	3.00	Cycle Inject Valve	
2			
3			
4			
5			
6			
-			

OK Cancel

Determining Duration of Gradient Time



Modify ACQUITY Binary Solvent Manager Instrument Method

Acquity Binary Solvent Manager UPLC[®] Run Time: 2.00 min

General | Data | Analog Out | Events

Solvents:

A1: 90%water/10% ACN

B2: Acetonitrile

Pressure Limits: Low: 0 psi High: 15000 psi Seal Wash: 5.0 min

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.600	5.0	95.0	Initial
2	30.00	0.600	5.0	95.0	11
3					
4					
5					
6					

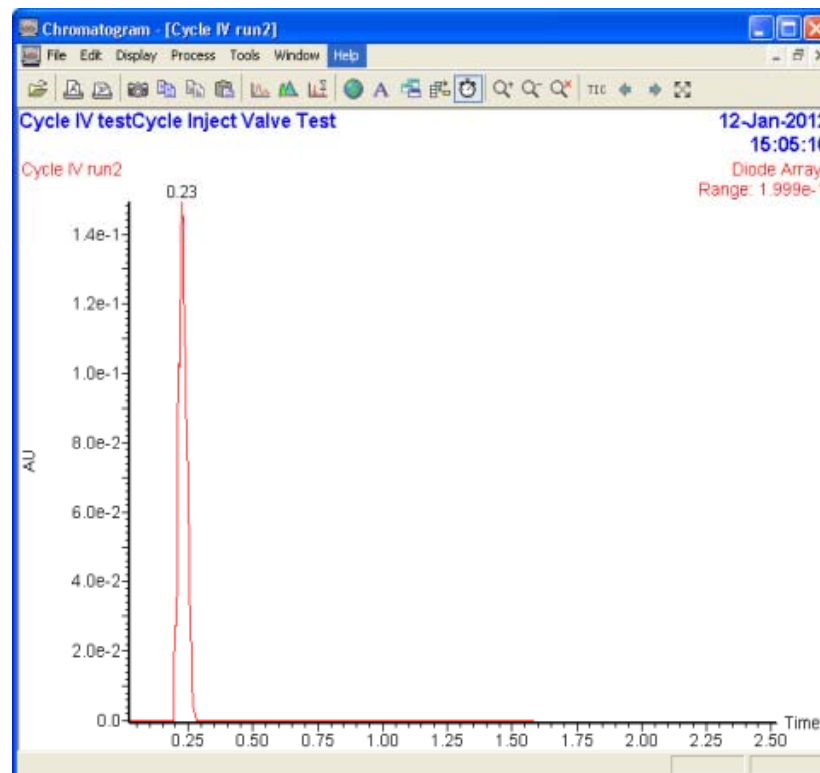
Gradient Start: ☒ At injection ☐ Before injection ☐ After injection

0 uL

Comment:

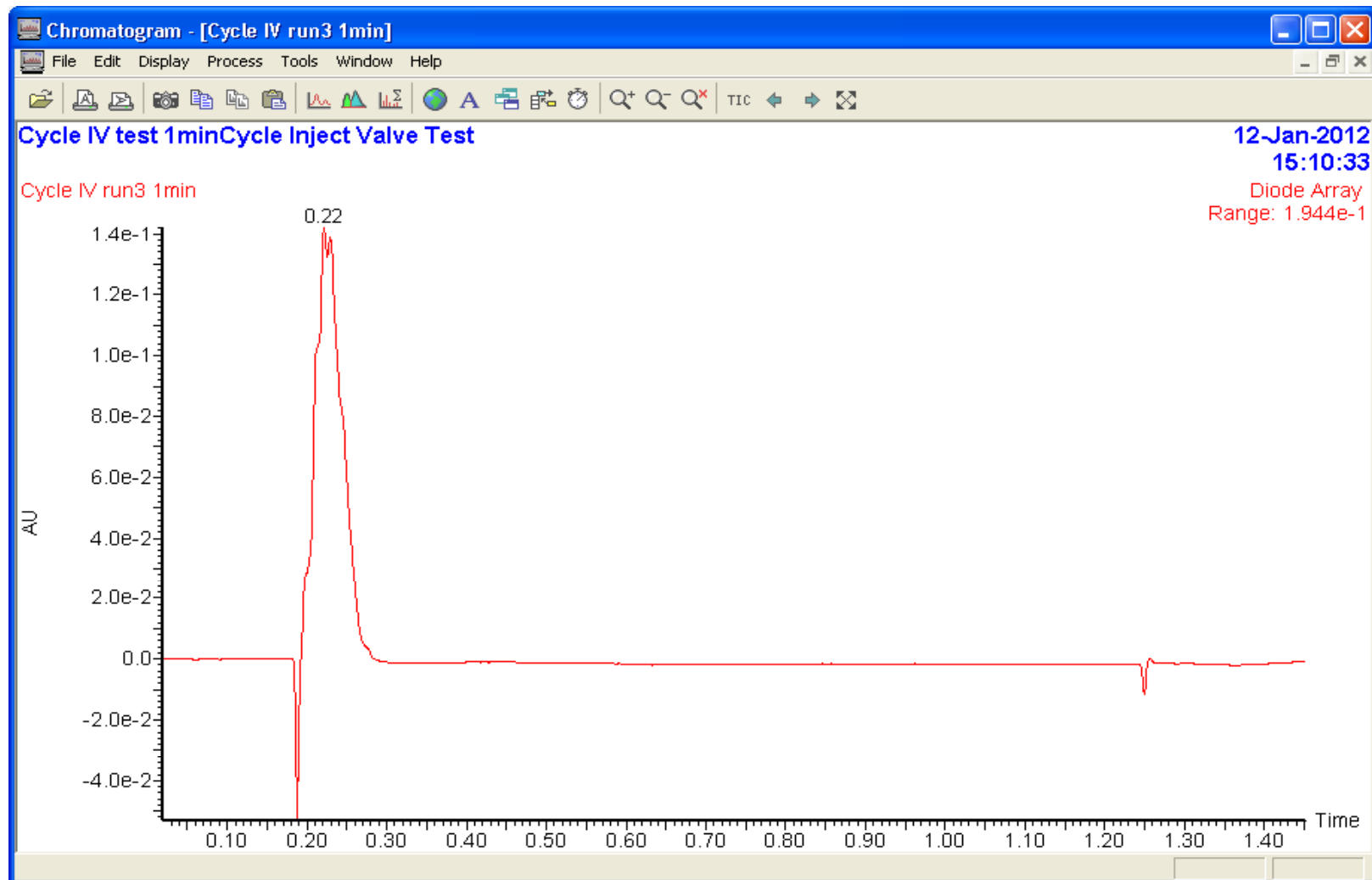
OK Cancel

Set up an isocratic method to run at the end conditions (highest percent organic) of your gradient method.



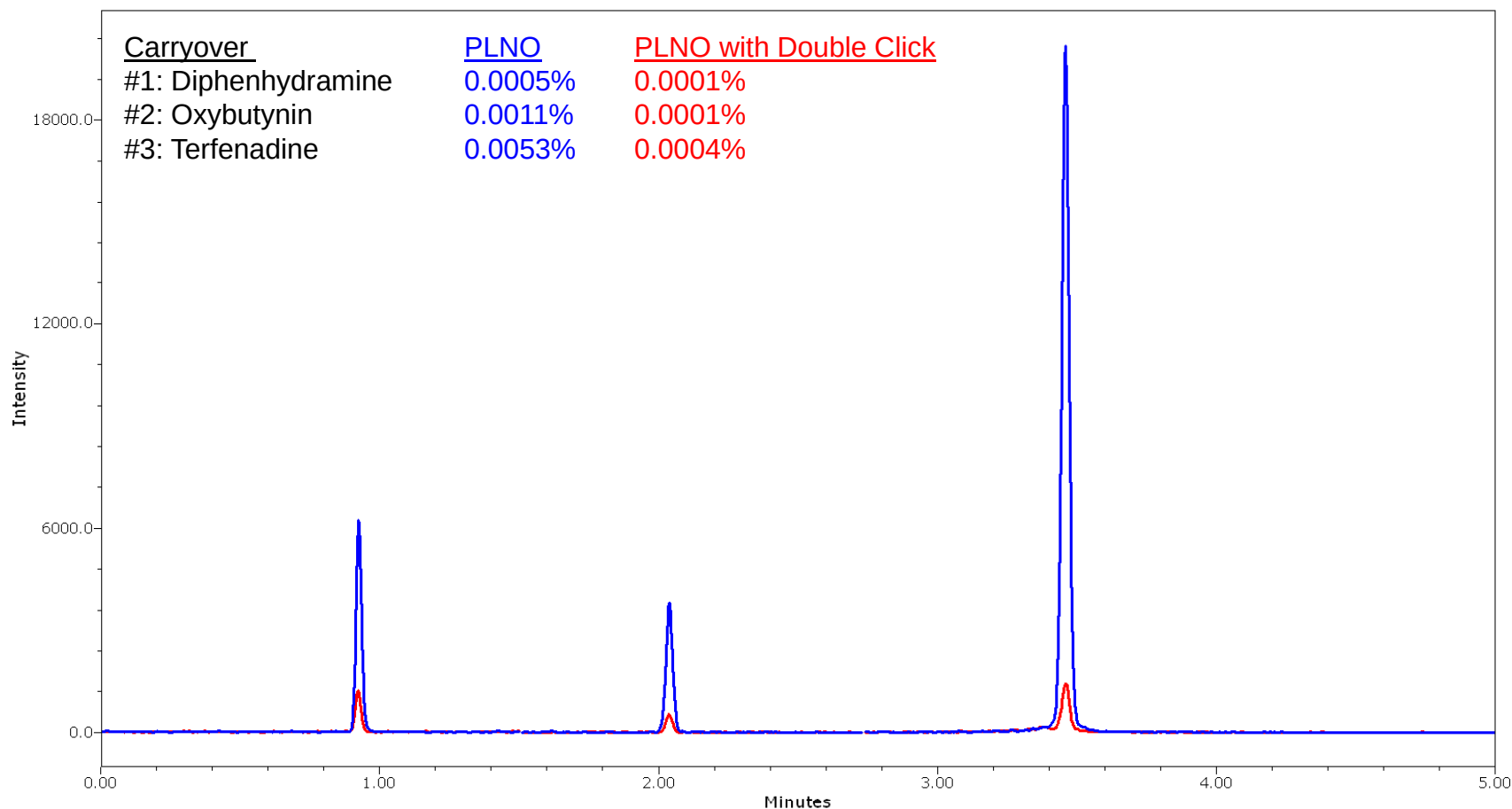
Inject your sample and determine the time it takes for all your compounds of interest to elute. If the organic is high enough, the compounds will most likely co-elute (as seen above). This would indicate the time you need to add to your end condition segment.

Evaluating Cycle Inject Valve Result



In this example, a Cycle Inject Valve event was set for 1 minute end condition conditions. at the time it takes for all your compounds of interest to elute. The compounds of interest co-elute at 0.22 min. A slight blip in the baseline can be seen at 1.22 min. indicating possible carryover of material between ports 1 and 2.

SM-FL: MS Carryover Improvement with 'Double Click'



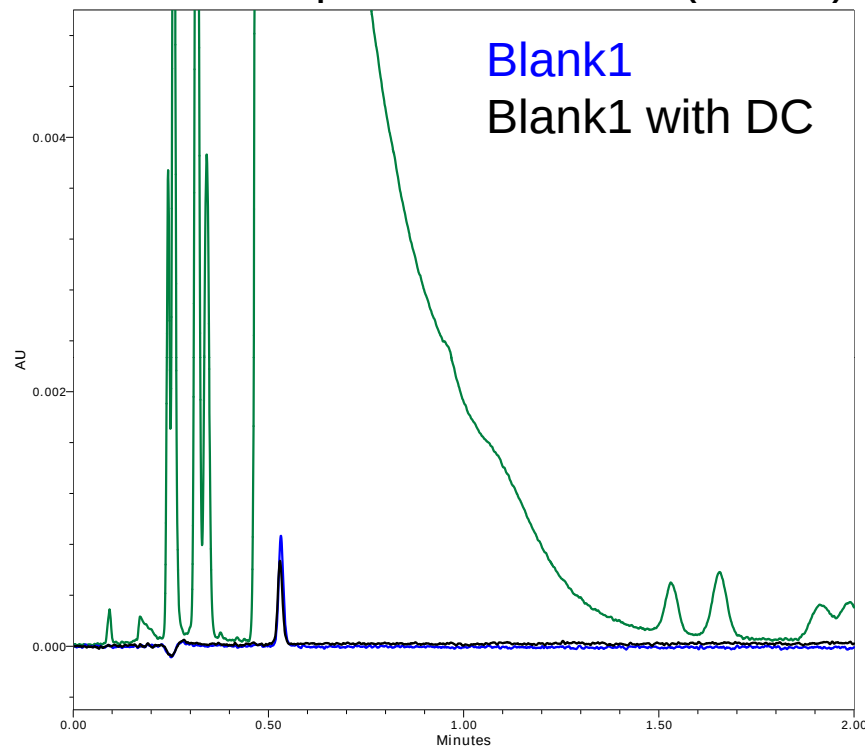
1st Blank Injection after High Concentration Standard PLNO

1st Blank Injection after High Concentration Standard PLNO with 'Double Click'

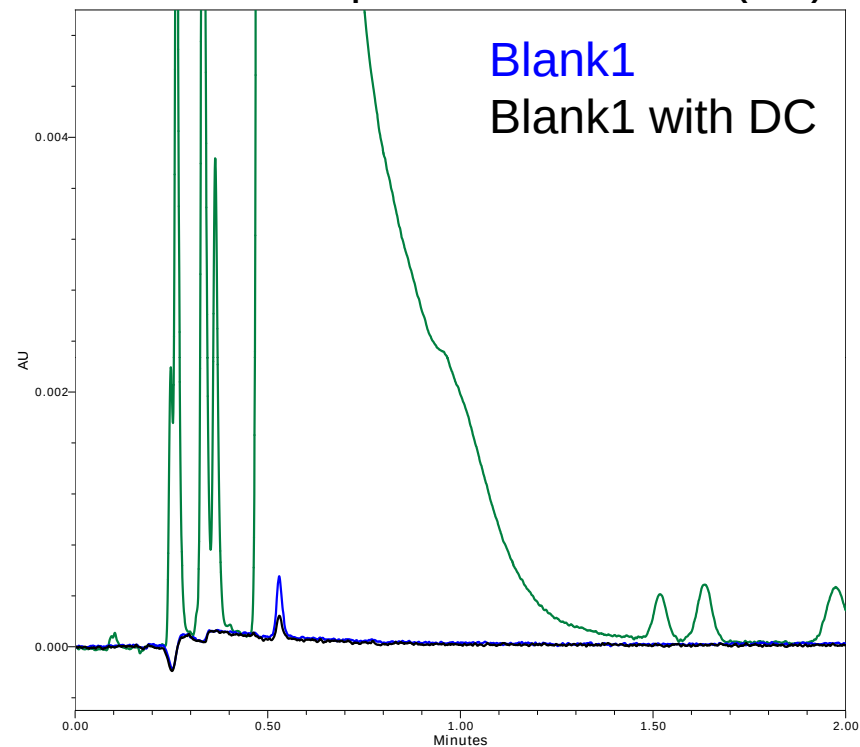
SM-FL: UV Carryover Improvement with 'Double Click' - Caffeine



Partial Loop Needle Overfill (PLNO)



Partial Loop Pressure Assist (PL)

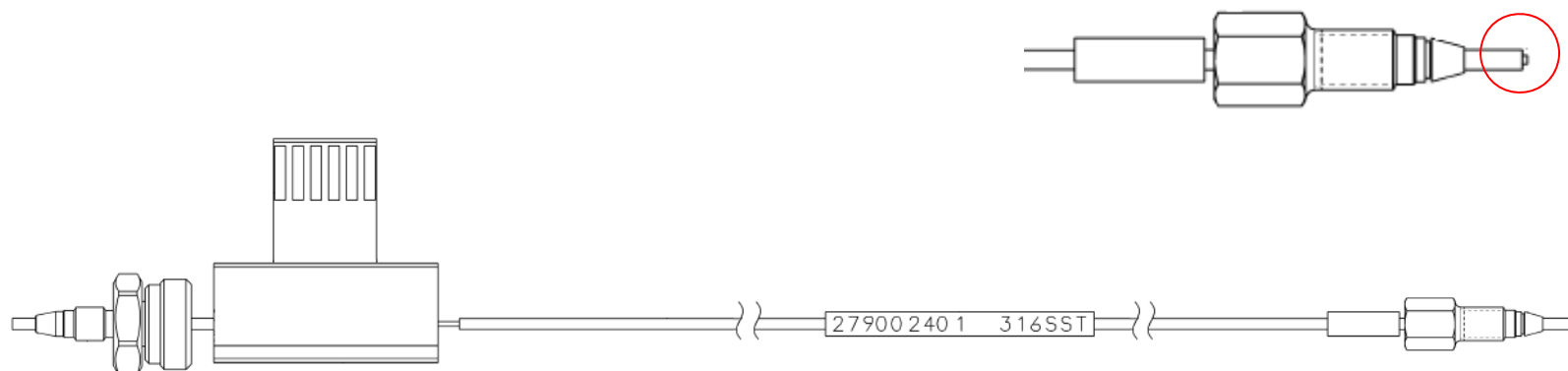
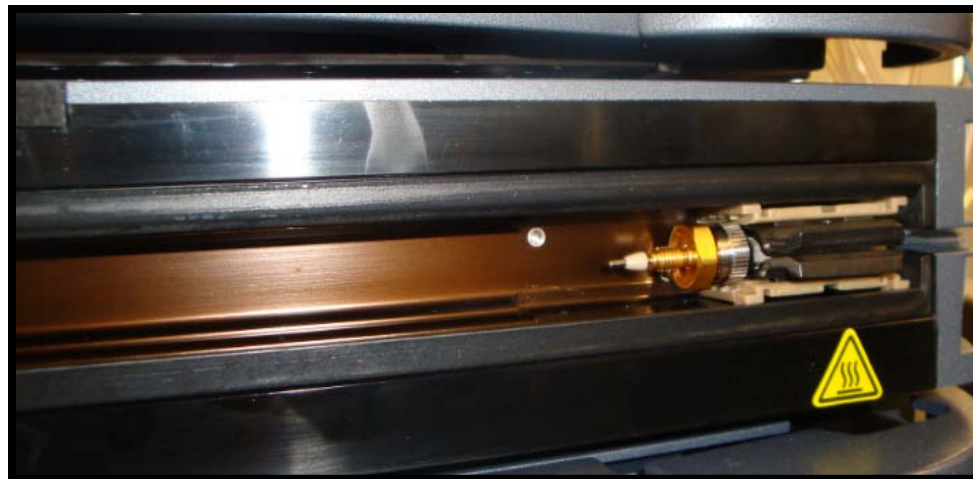


Injection Mode	Double-Click	Carryover
PLNO	No	0.00100%
PLNO	Yes	0.00062%
PL	No	0.00064%
PL	Yes	0.00024%
FTN	--	0.00034%

NEW Active Pre-Heater



- 18,000 PSI capable
- Improved Dispersion
 - 0.003" ID Tubing
 - Protruded end
 - Modified “V” Detail
 - Positive contact point



ACQUITY UPLC I-Class System

Optical Detection



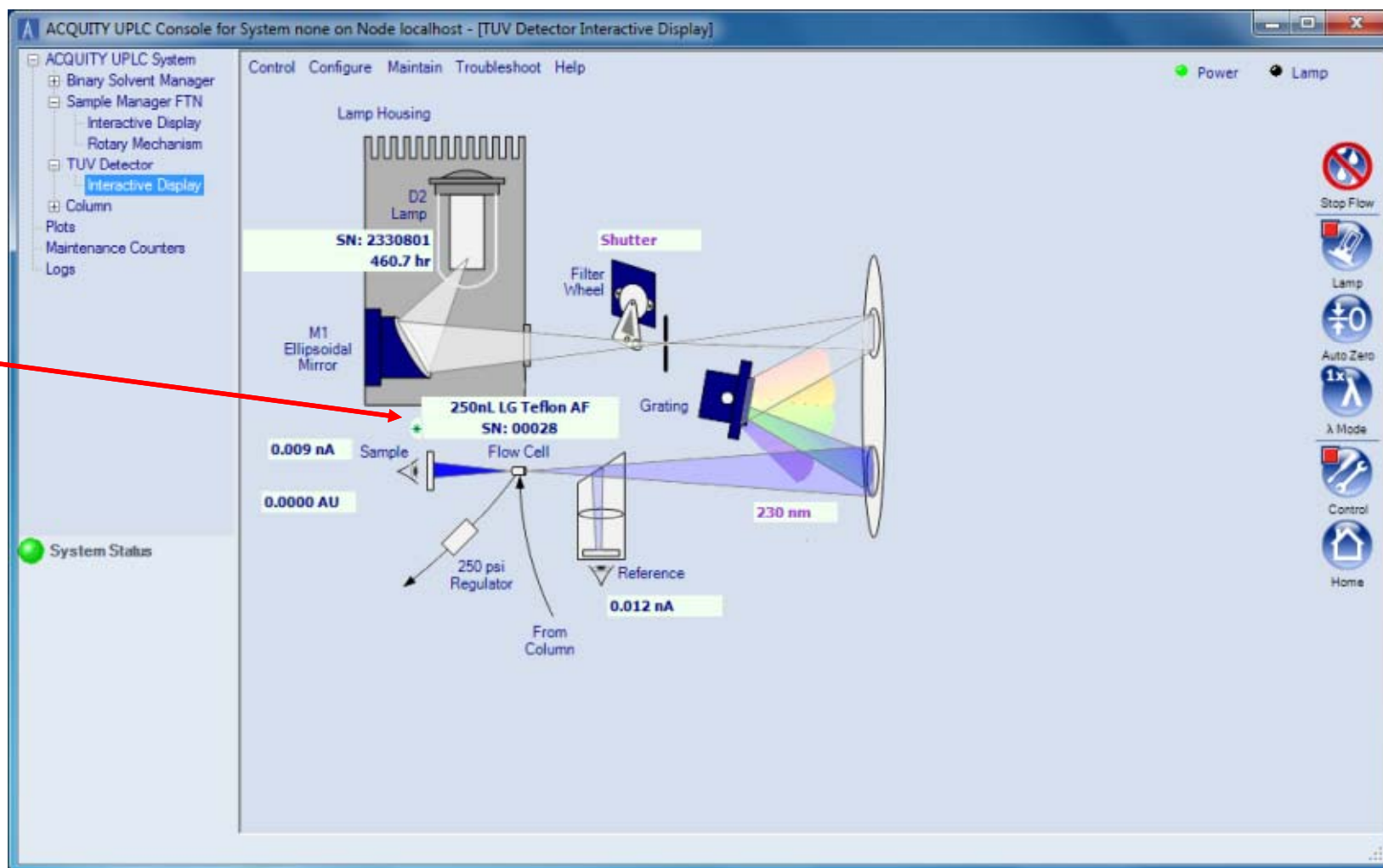
- What's New?
 - 500nL flow cell has been optimized for reduced dispersion
 - Flow cell body is the same
 - Inlet and outlet tubing i.d. has been reduced
 - .0025" ID inlet PEEK tubing
 - .0035" ID outlet PEEK tubing
 - 250nL / 183 μ m version for 1mm chemistry
 - Reduced flow rate range
 - still being characterized – information available via shortly.



TUV Interactive Display



Flow Cell type
and S/N is read
from chip



New TUV Preferences Menu



TUV Preferences

Shutter Control

Close shutter:

☒ After 10 minutes of inactivity

☐ Never

Optics Temperature Stabilization

Setting:

☒ Normal temperature

☐ Elevated temperature

☐ Maximum cooling

Component Identification Requirement

Require for operation:

☐ Lamp resident identification

☐ Flow cell resident identification

OK Cancel

You can set the type of environment that is present in your lab, based on a general temperature rating.

In the Optics Temperature Stabilization field, you can specify the following settings:

Normal temperature - Use this setting if the temperature in your lab is normal room temperature.

Elevated temperature - Use this setting if the temperature in your lab is slightly higher than normal room temperature.

Maximum cooling - Use this setting if the temperature in your lab is consistently much higher than normal room temperature.

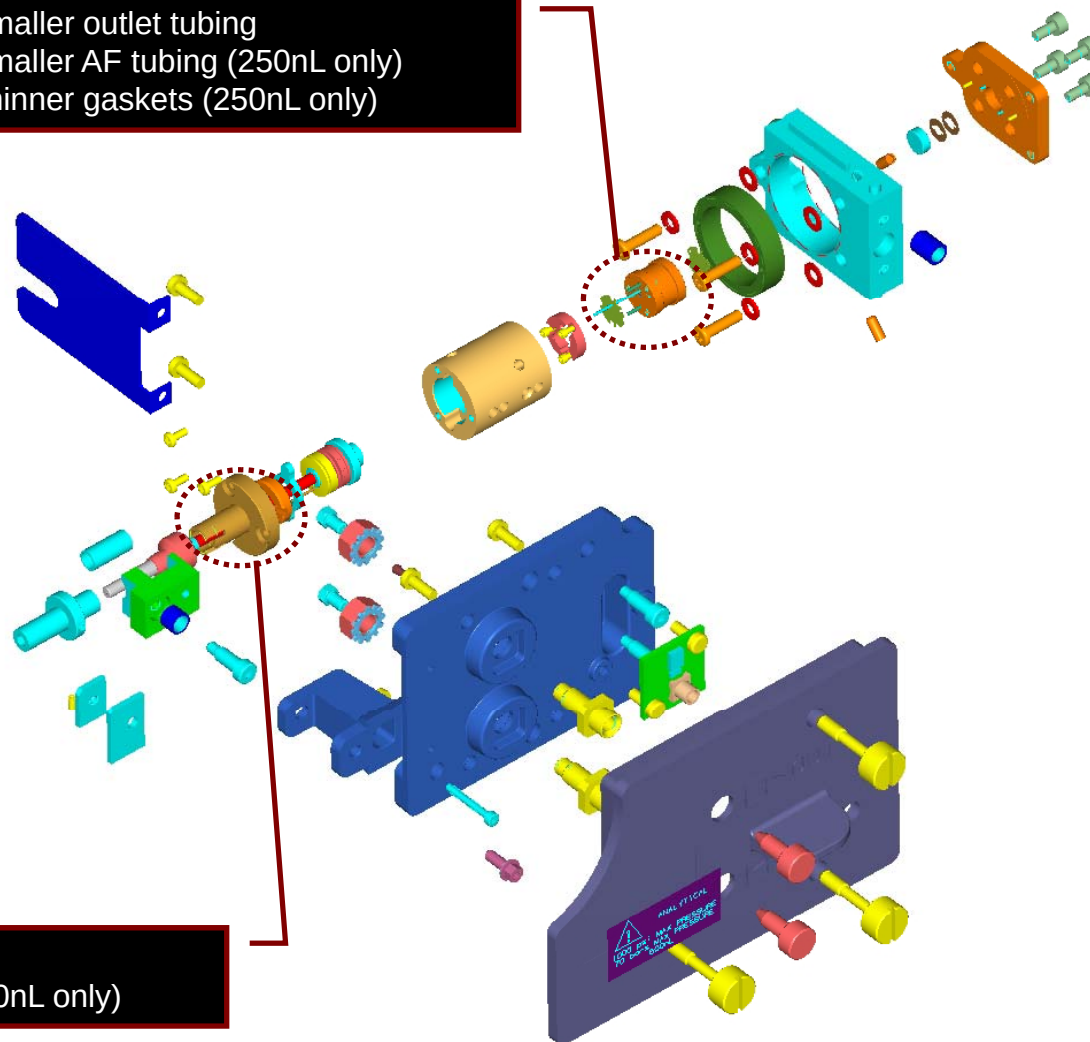
Require for Operation

If the fields are checked, it would require the presence of the electronic Lamp and/or Flow Cell serial numbers to be present to start a run.

I-Class PDA Flow Cell



- Smaller outlet tubing
- Smaller AF tubing (250nL only)
- Thinner gaskets (250nL only)



- Smaller inlet tubing
- Thinner gaskets (250nL only)

Current and New Flow Cell Configurations



Flow Cell	Pathlength	Teflon AF ID	Inlet ID	Outlet ID
Current Analytical	10 mm	240 μ m	.005"	.005"
Low Disp Analytical	10 mm	240 μ m	.0025"	.0035"
New 250nL	10 mm	183 μ m	.0025"	.0025"

Volume (nL) & Bandspreading Improvements



Flow Cell	Inlet Tube Volume	Optical Volume	Outlet Tube Volume	Total Volume
Current Analytical	1078	448	1207	2733
Low Disp Analytical	270	448	591	1309
New 250nL	270	263	302	835

Flow Cell	Band Spread	Improvement over Analytical
Standard Analytical	6.38 μ L	--
Analytical with 0.0025"	5.78 μ L	9.4%