

ACQUITY UPLC® Systems Course

Introduction and System Overview

v. 20072010

Course Overview

- ❖ Full coverage of the ACQUITY Product line
 - ❖ ACQUITY H-Class system as an introduction
 - ❖ ACQUITY I-Class
 - ❖ As it differs from the H-Class systems
 - ❖ Its similarities with ACQUITY Classic
 - ❖ ACQUITY Classic as it differs from H-Class and I-Class
 - ❖ Add-on components (eg. Sample Organizer, CM-A)
 - ❖ ACQUITY Detectors
- ❖ Workbook based
 - ❖ Brief lectures to give an overview and then follow the workbook
 - ❖ Test each system before and after performing all service procedures
 - ❖ Taking the modules apart is encouraged as long as you can successfully put it back together
 - ❖ Follow the procedures on the portal as much as possible

Outline for this Presentation

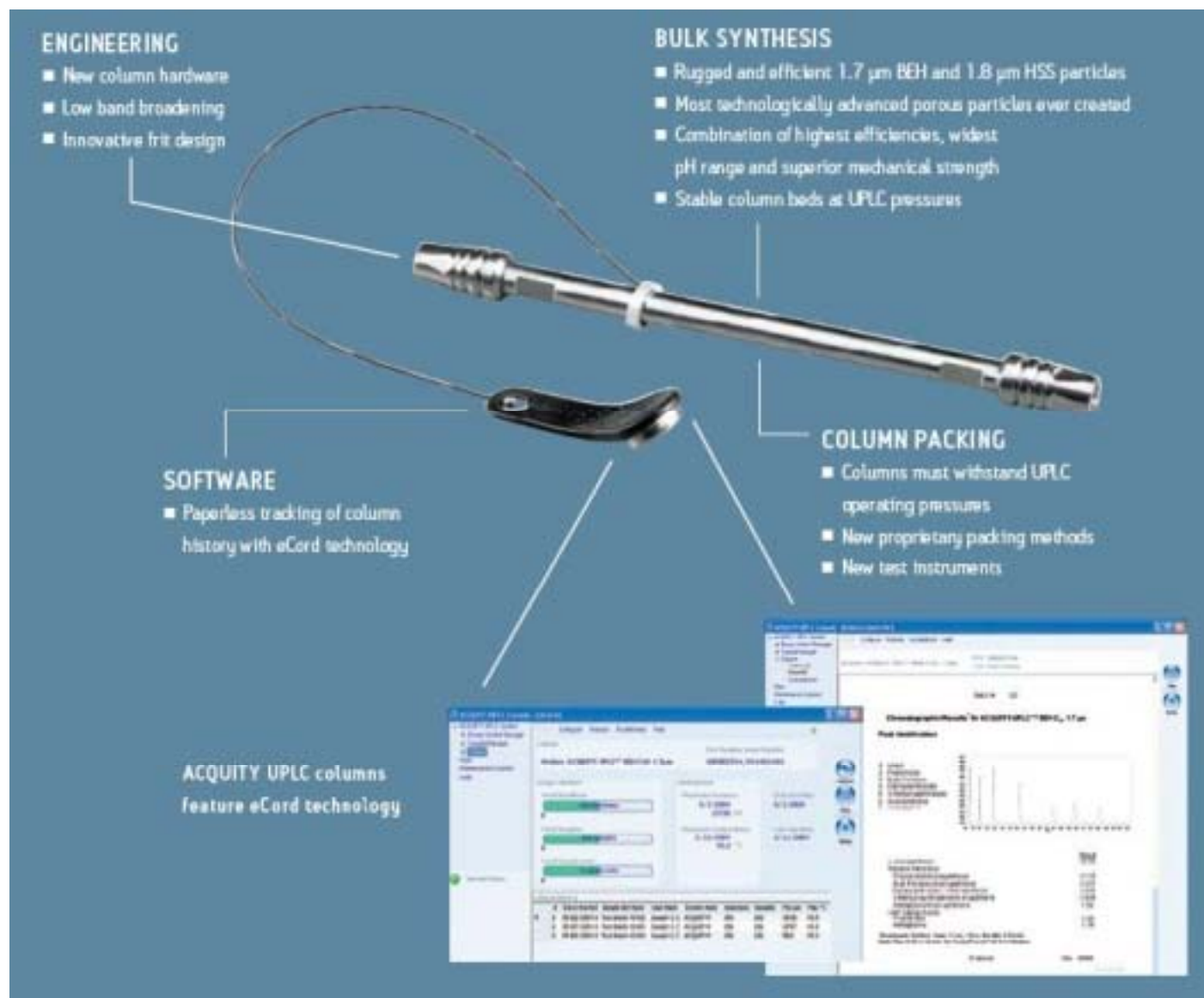
- ❖ Brief ACQUITY Chemistry Overview
- ❖ Overview of current ACQUITY System Components
 - ❖ ACQUITY H-Class
 - ❖ ACQUITY I-Class
 - ❖ ACQUITY Classic



Chemistry Overview

ACQUITY UPLC® Service Training Introduction

ACQUITY Column Engineering



The image displays an ACQUITY UPLC column and its associated software interface. The column is a long, thin, metallic tube with a coiled section at one end. It is shown against a dark blue background. The software interface is a screenshot of the ACQUITY UPLC Manager, showing various parameters and a chromatogram.

ENGINEERING

- New column hardware
- Low band broadening
- Innovative frit design

BULK SYNTHESIS

- Rugged and efficient 1.7 μm BEH and 1.8 μm HSS particles
- Most technologically advanced porous particles ever created
- Combination of highest efficiencies, widest pH range and superior mechanical strength
- Stable column beds at UPLC pressures

SOFTWARE

- Paperless tracking of column history with eCard technology

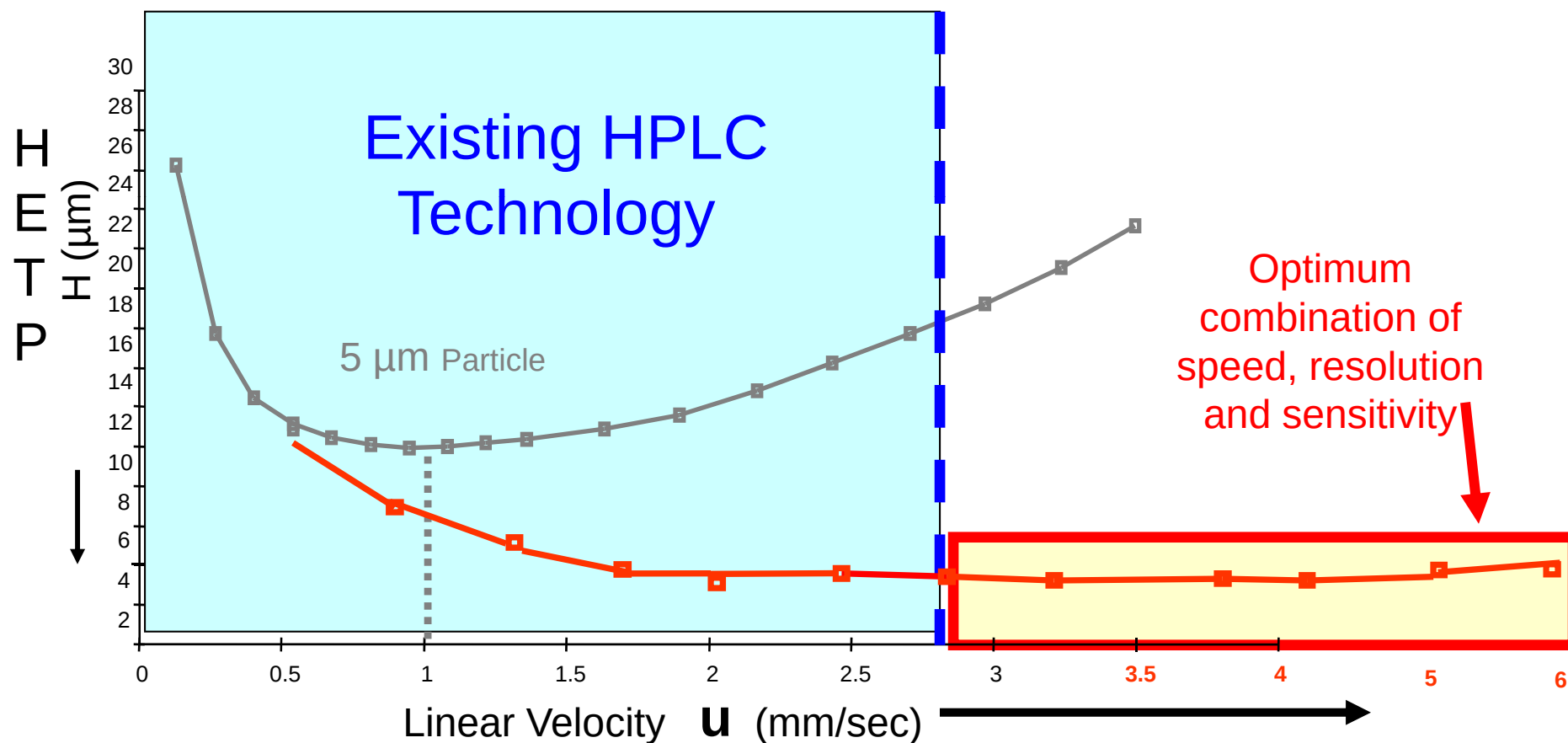
COLUMN PACKING

- Columns must withstand UPLC operating pressures
- New proprietary packing methods
- New test instruments

ACQUITY UPLC columns feature eCard technology

*From the ACQUITY UPLC Columns Brochure

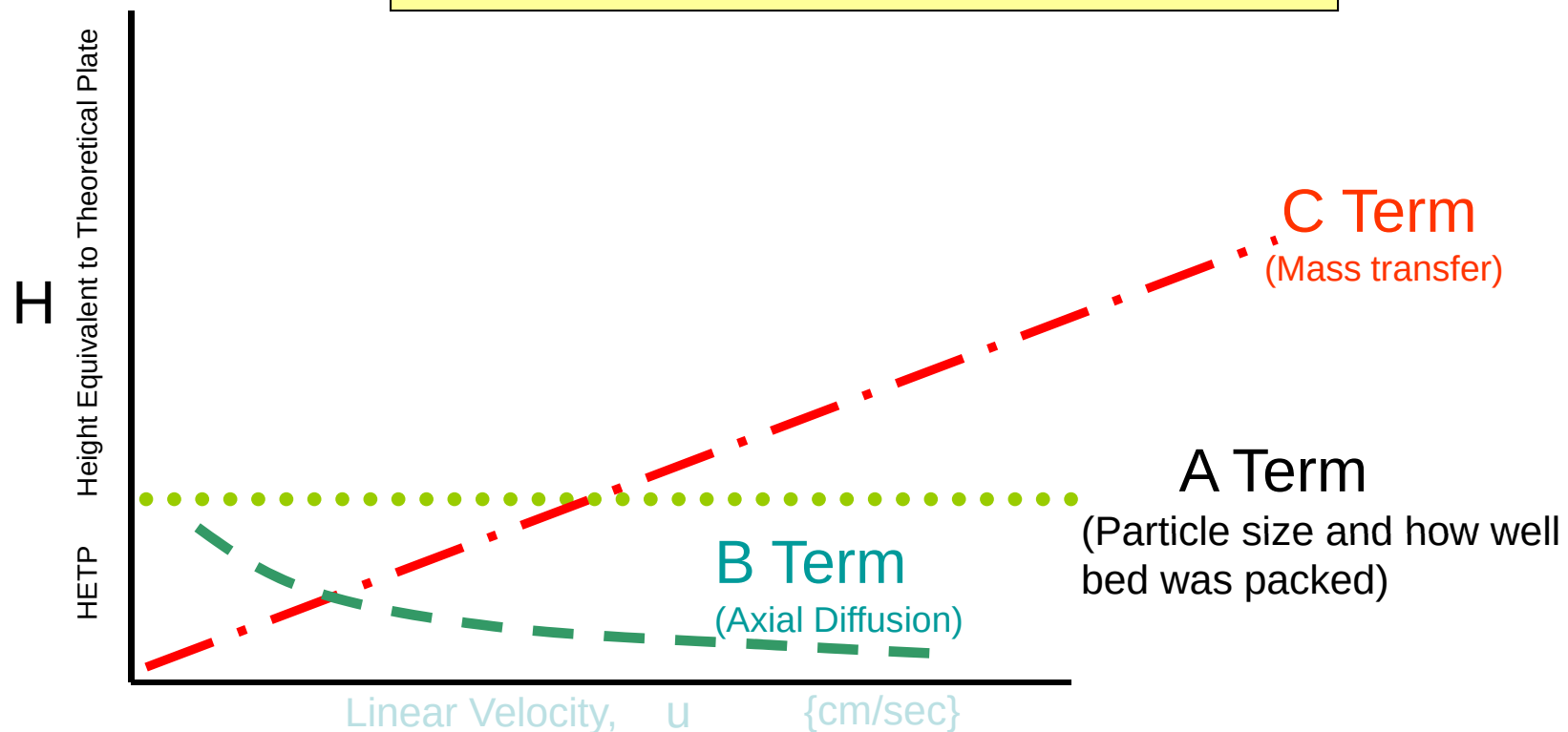
Smaller Particles - The enabler of productivity



Particle Size and Flow Rate - Van Deemter Equation

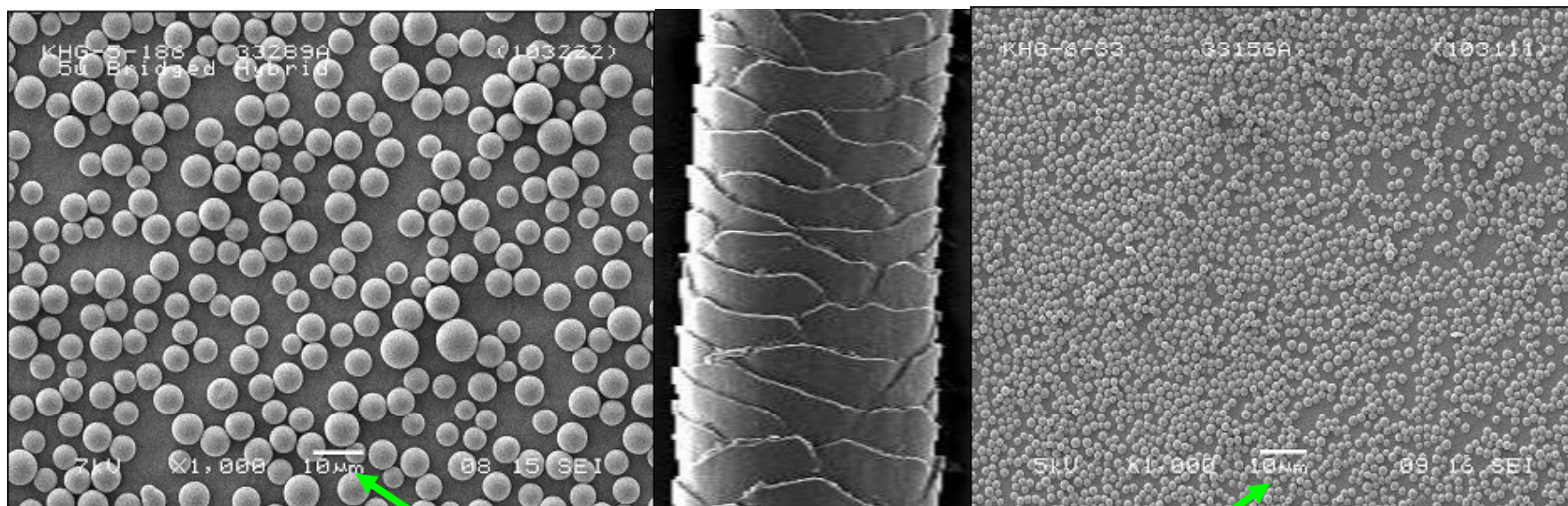
A term + B term + C term

$$\bar{H} = a(dp) + \frac{b}{u} + c(dp)^2u$$



A Term- Particle Size and Packing Efficiency

60 μm Human Hair
(very fine hair)



5 μm
Analytical Particles
(can fit 12 across hair)

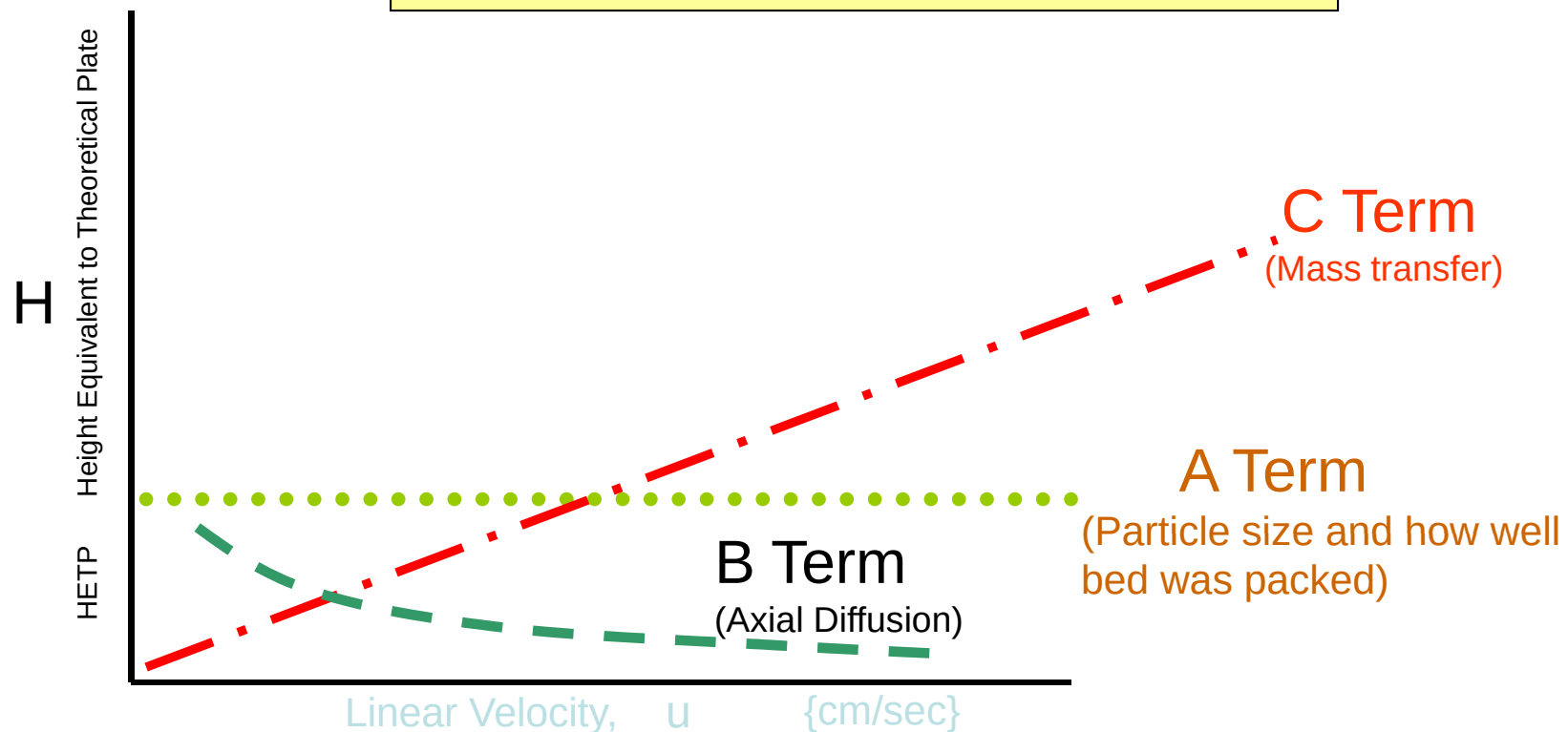
Images are on
same scale
(Bar = 10 μm)

1.7 μm
ACQUITY UPLC® Particles
Optimal Particle Size
Distribution
(leading to lower A term)
For Max Efficiency
at a given Pressure

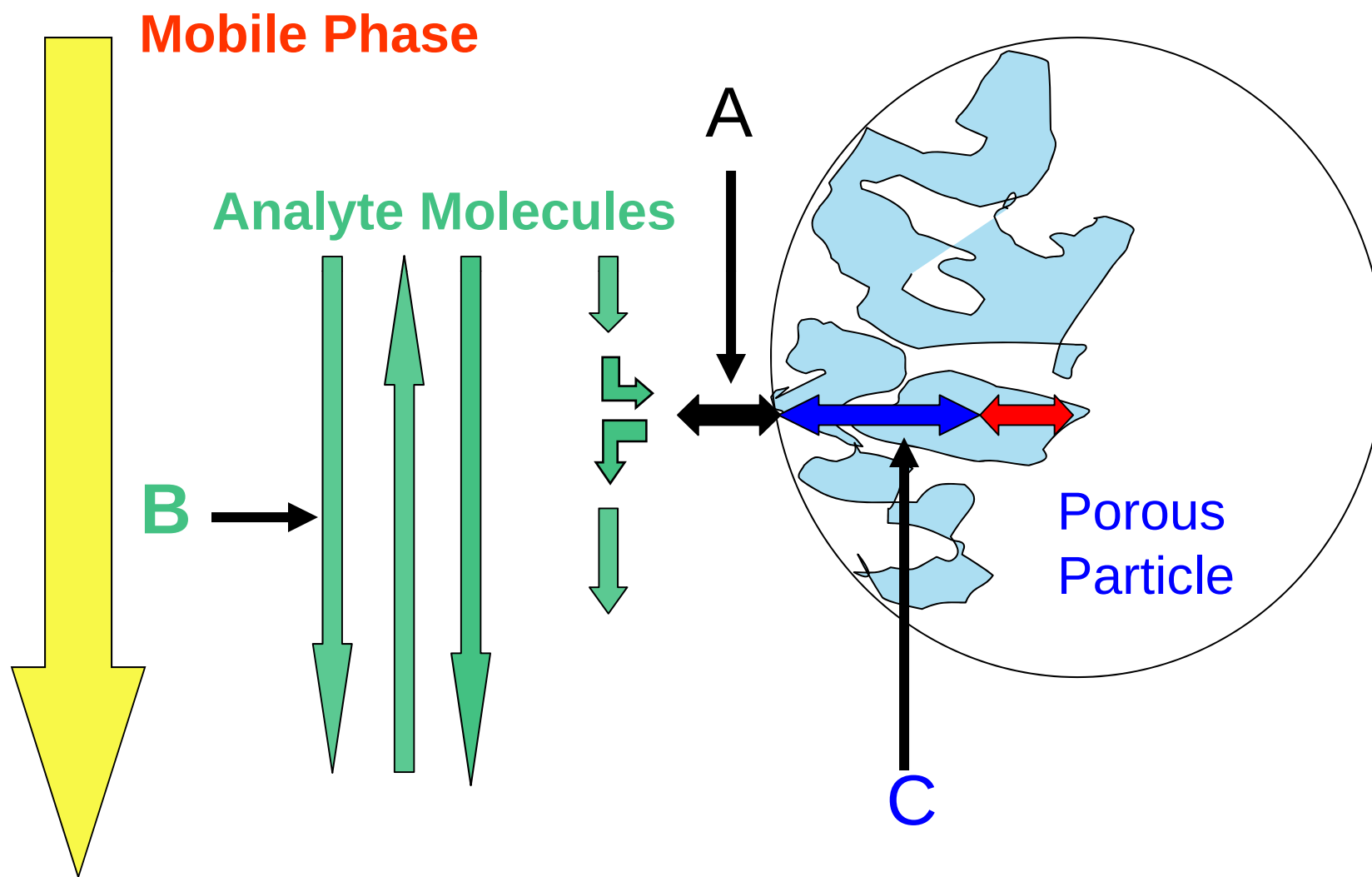
Particle Size and Flow Rate - Van Deemter Equation

A term + B term + C term

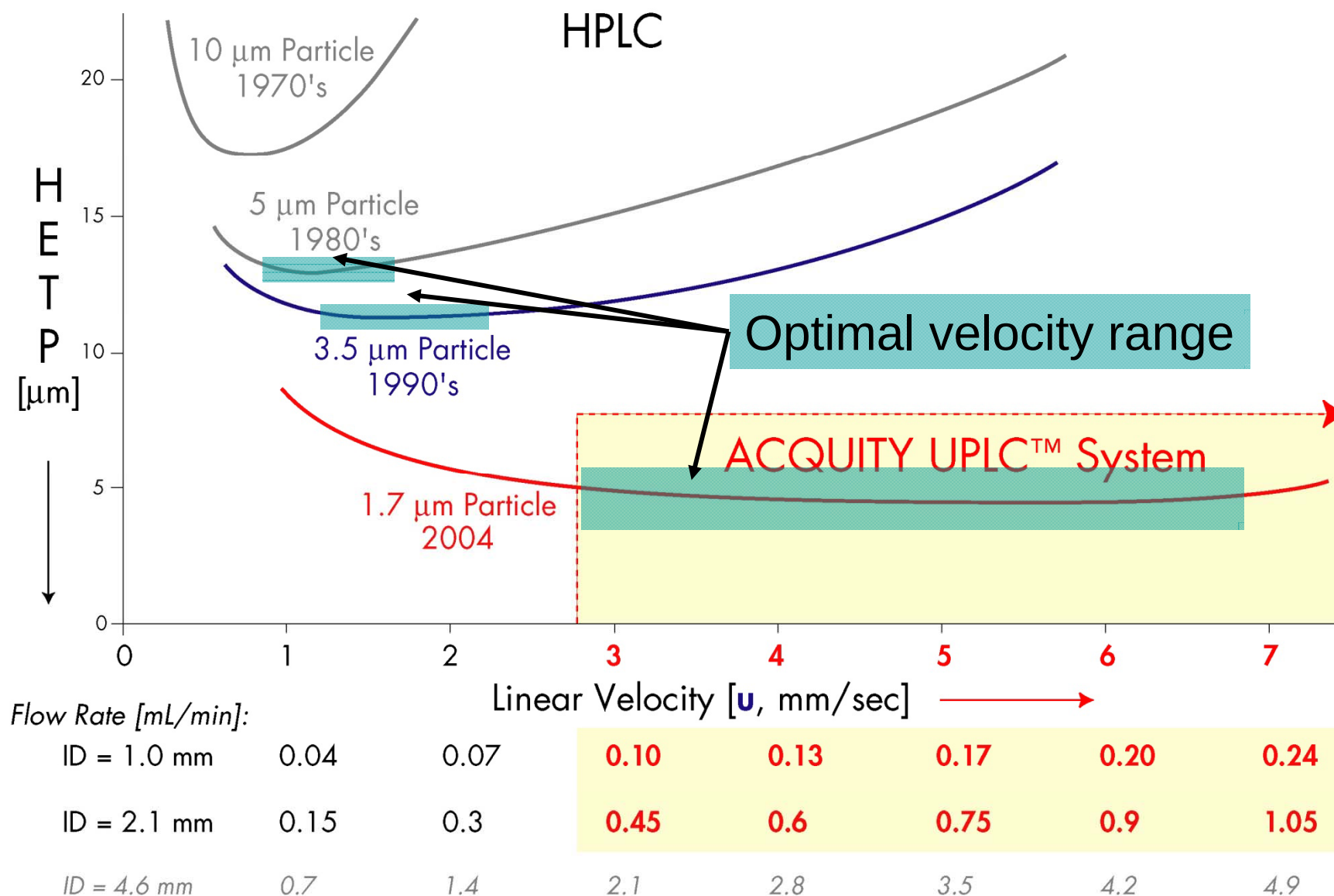
$$\bar{H} = a(dp) + \frac{b}{u} + c(dp)^2u$$



Chromatography Principles - Mass Transfer / Diffusion



Smaller Particles - The Enabler of Productivity



Summary of Key System Attributes

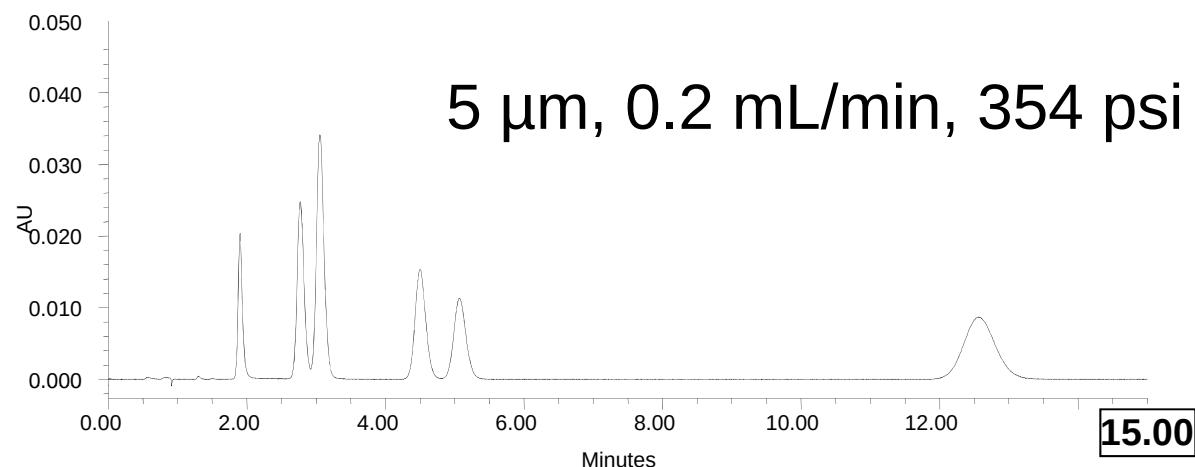
Constant column length

A term + B term + C term

$$H = a(dp) + \frac{b}{u} + c(dp)^2u$$

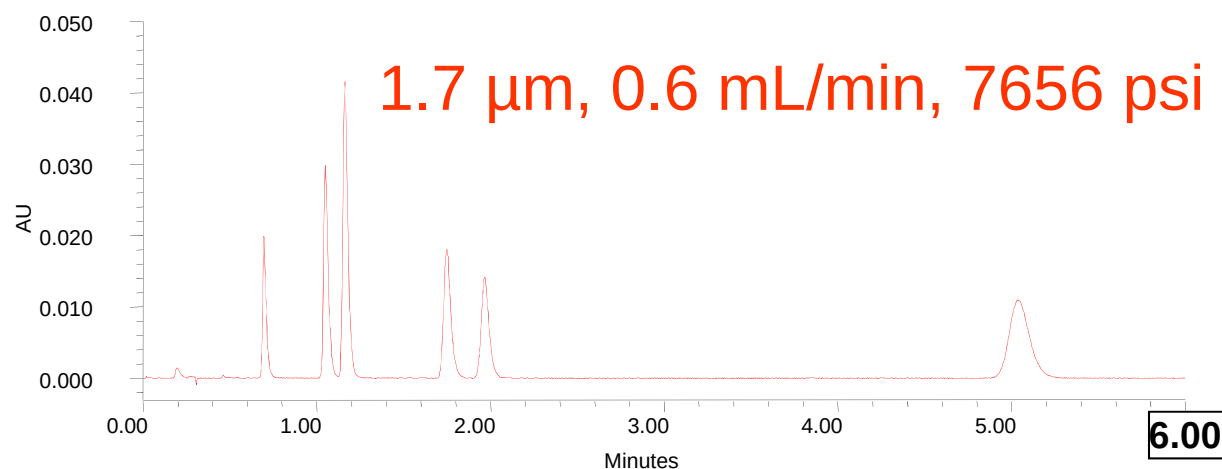
	Resolution Improvement	Speed Improvement	Sensitivity Improvement
5 to 1.7 µm particles	1.7X	3X	1.7X
3 to 1.7 µm particles	1.3X	2X	1.3X

Fixed Column Length: More resolution faster



Theory

1.7X Resolution
3X Faster
1.7X Sensitivity
27X Pressure

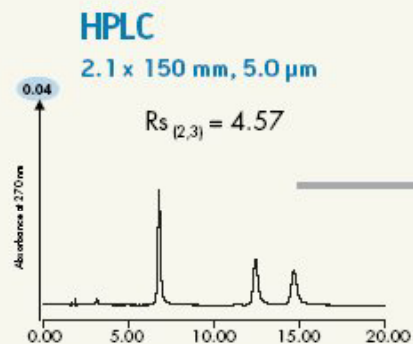


Actual

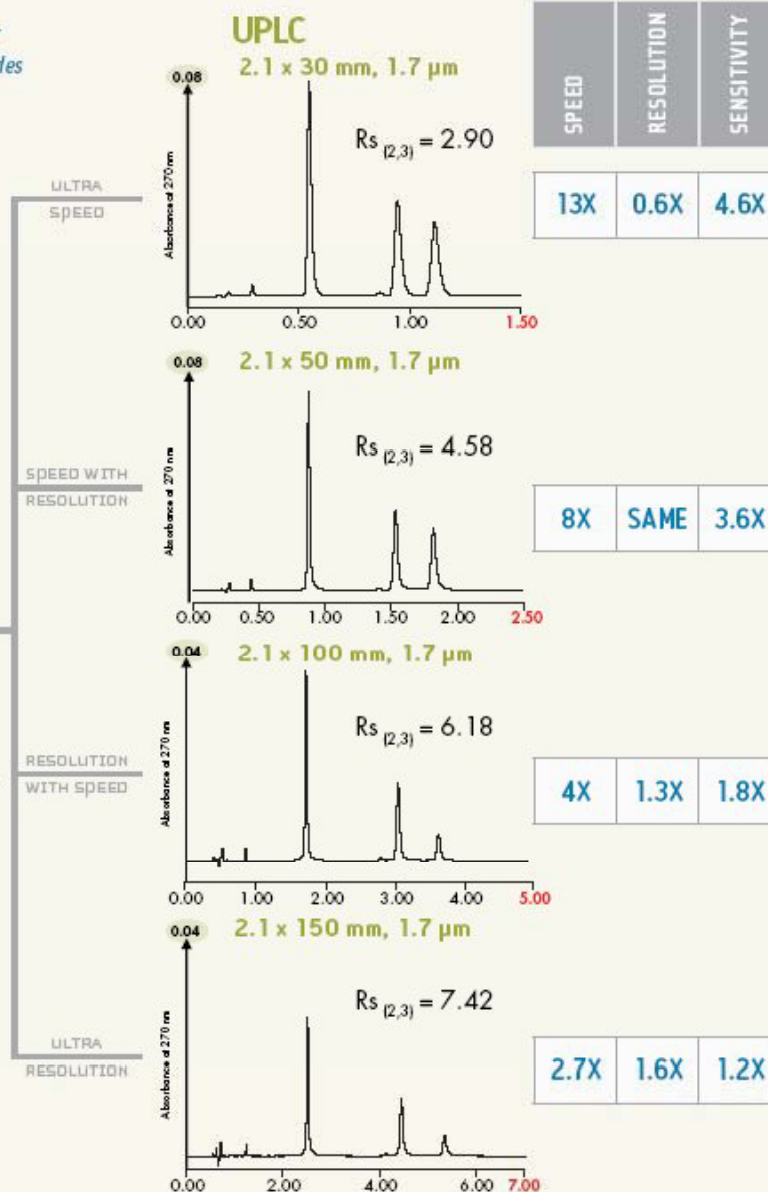
1.5X Resolution
2.6X Faster
1.4X Sensitivity
22X Pressure

Speed, Resolution, Sensitivity Options

Whether your objective is maximum speed or maximum resolution, UPLC technology provides you with the flexibility to achieve both.



Compounds:
1. Quercetin
2. Koenigseck
3. Isohamnol



*From the ACQUITY UPLC Columns Brochure

Column Length VS Separation Difficulty

UPLC Column Length	l/dp^*	Efficiency (N)	Separation Type
30 mm	17,600	5,875	Easy
50 mm	29,400	11,750	Moderately Challenging
100 mm	58,800	23,500	Difficult
150 mm	88,200	35,000	Extremely Difficult

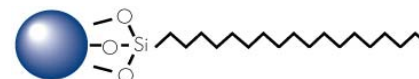
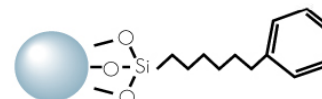
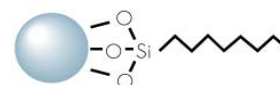
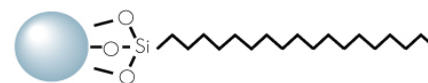
Choosing a UPLC column based upon the relationship between UPLC column length, length to particle size ratio (l/dp), efficiency (N) and separation type.

* $dp = 1.7 \mu m$

*From the ACQUITY UPLC Columns Brochure

ACQUITY UPLC® Column Chemistries

- **BEH C18**
 - Trifunctionally Bonded C₁₈
 - Proprietary Endcapping
 - Widest pH range
- **BEH C8**
 - Trifunctionally Bonded C₈
 - Proprietary Endcapping
 - Widest pH range
- **BEH Shield RP18**
 - Monofunctionally bonded
 - Embedded polar group
- **BEH Phenyl**
 - Trifunctionally Bonded C₆ Phenyl
 - Proprietary Endcapping
- **BEH HILIC**
 - Unbonded BEH particle
 - HILIC for very polar bases
- **HSS T3**
 - T3 Bonding & Endcapping
 - Superior Polar Compound Retention



Method Transfer Options using the ACQUITY Calculator

ACQUITY UPLC Columns Calculator - Untitled

File Edit Help

Analytical Factors

☒ **Isocratic** Molecular Weight: **0-100** Da Column Temperature: **30** °C Retention Factor (k'): **8** 

☐ Gradient

HPLC (Binary) ▼ **From HPLC** → **To UPLC** UPLC (BSM) ▼

From HPLC **L/dp = 30,000**

Column Length (L): **150** mm
Column Diameter: **4.6** mm
Particle Diameter (dp): **5** µm
Injection Volume: **10** µL

To UPLC **L/dp = 29,412**

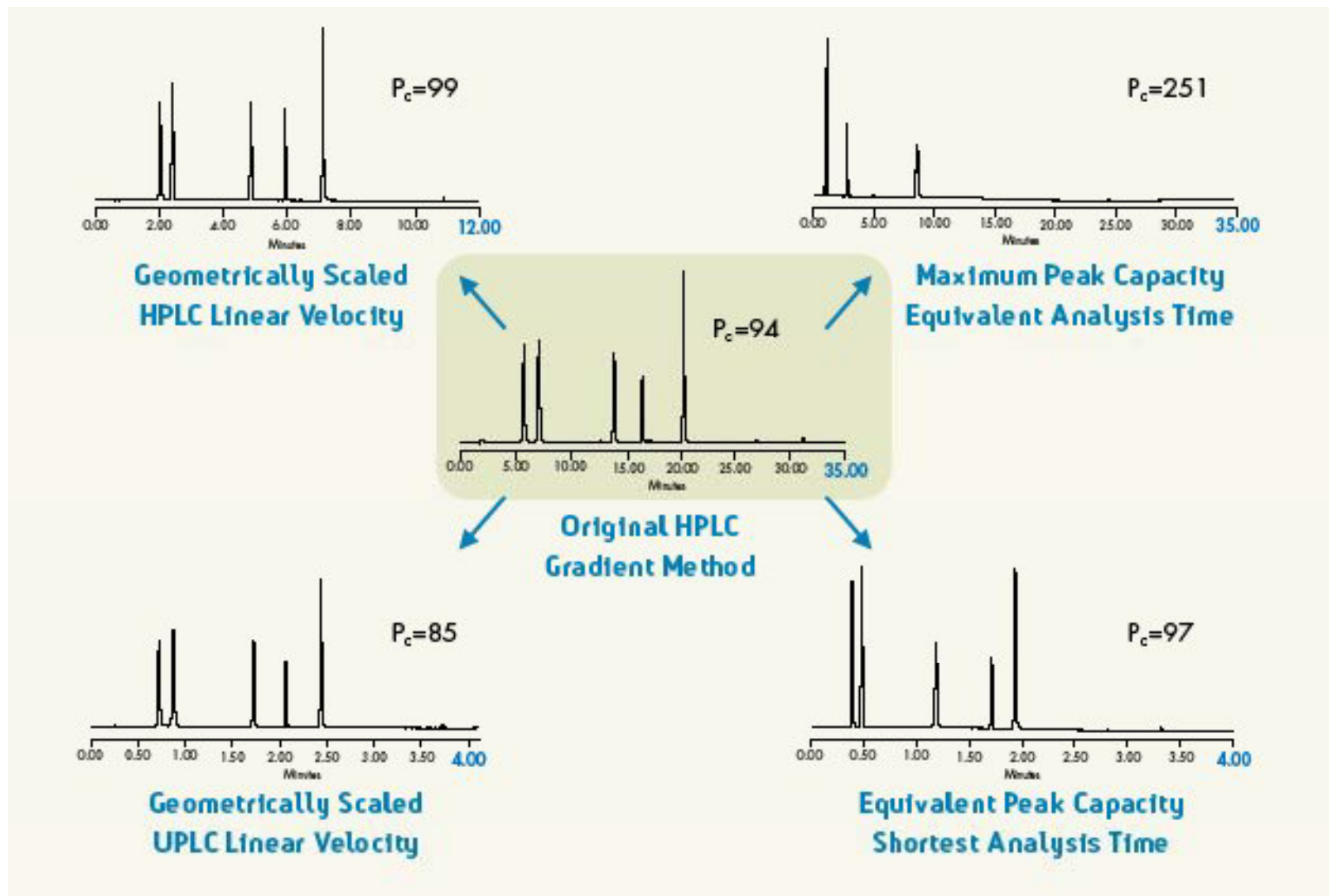
Column Length (L): **50** mm
Column Diameter: **2.1** mm
Particle Diameter (dp): **1.7** µm
Maximum Pressure: **14997** psi

Original Isocratic Flow
862 psi

Organic Mix
Acetonitrile: **100.0** % Methanol: **0.0** %

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Organic)	Column Volumes
▶ 1	Initial	1.00	100.0	0.0	--

Method Transfer Options using the ACQUITY Calculator





ACQUITY H-Class I-Class Systems



Rev. 2.0

4/28/2010

ACQUITY H-Class System

- Allows customers to move up to UPLC while making it easier to run existing HPLC methods on the same instrument
- The ACQUITY H-Class System consists of the following:
 - Quaternary Solvent Manager (QSM)
 - Four solvent low pressure gradient mixing
 - Supports UPLC Pressures
 - Low gradient delay volume
 - SM-FTN (Sample Manager-Flow Through Needle)
 - One injection mode- direct inject type
 - Much simpler- Just specify injection volume
 - Excellent recovery and precision
 - CH-A
 - High temperature column heater with active pre-heating
 - CM-A
 - Dual Column Manager for up to 4 columns
 - Up to 6 columns are supported with CM-Aux slave units

ACQUITY H-Class System

CH-A
High Temperature Column Heater
Active Pre-Heating

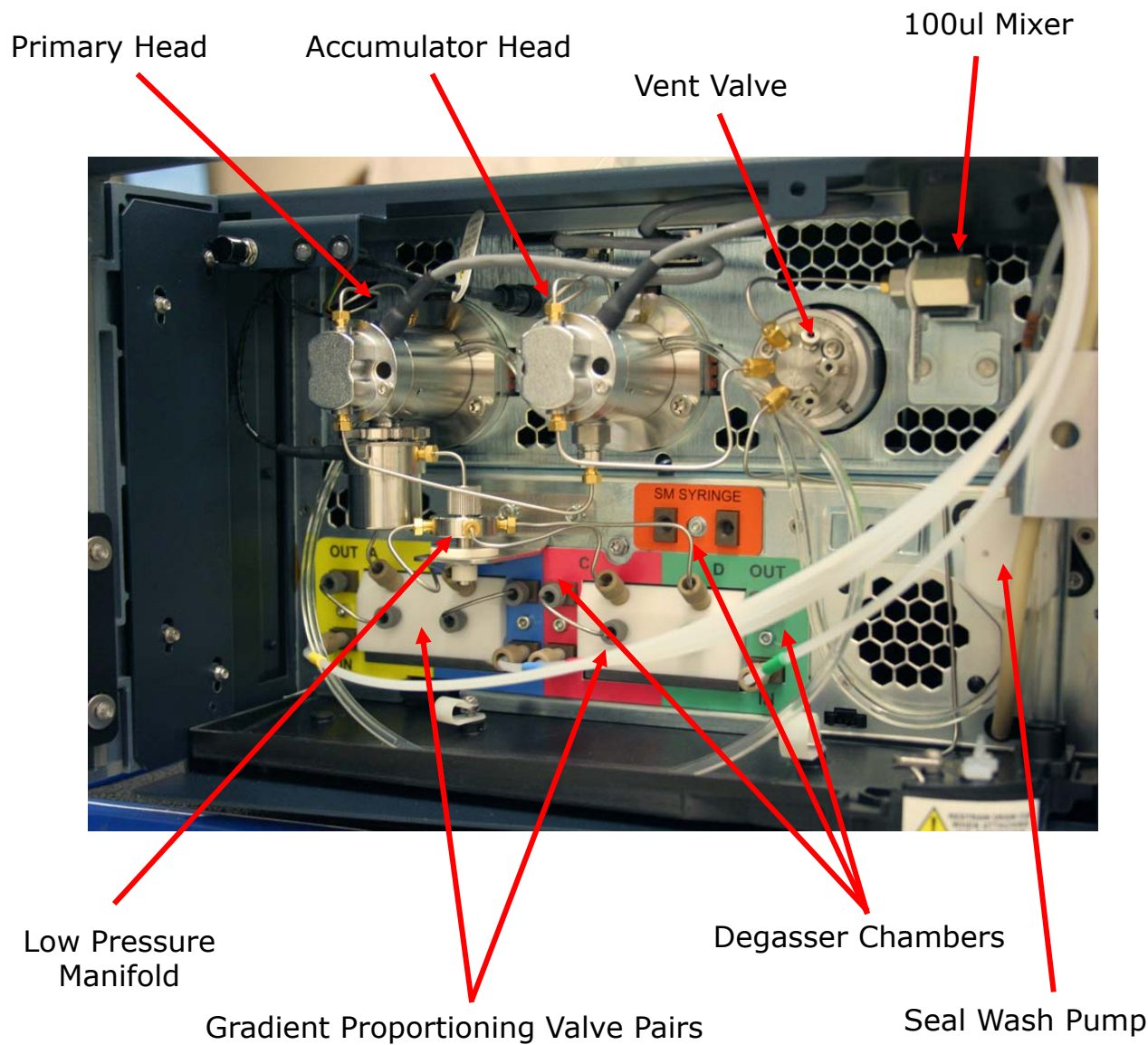
QSM
Quaternary Solvent Manager



Detectors
TUV, PDA eλPDA, FLR, ELSD

SM-FTN
Sample Manager-Flow Through Needle

H-Class QSM- Low Pressure Mixing



Common Platform SM- (FTN or FL)

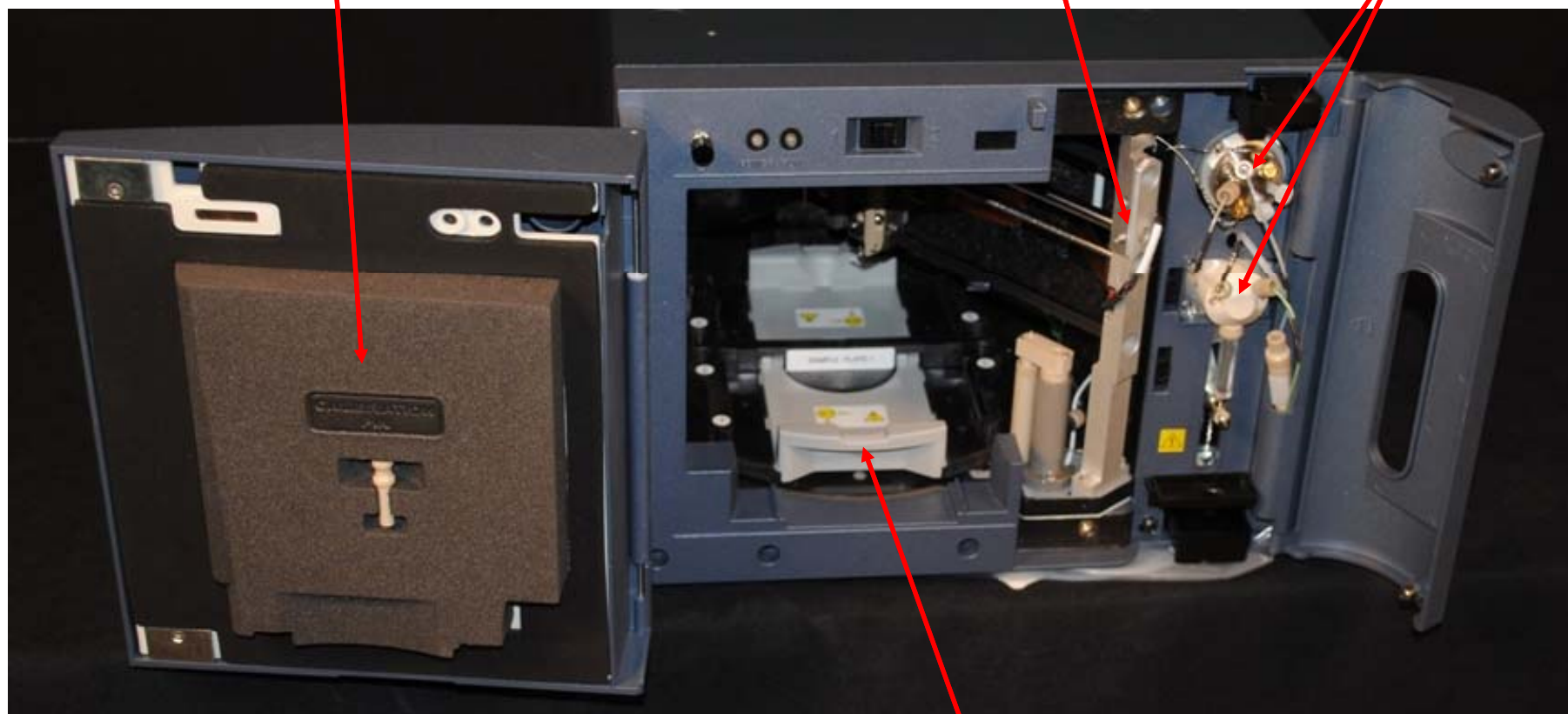


SM- Common Component Overview

Sample Compartment Door
Insulating Foam

R-Carriage
Assembly

Inject Valve and
SM Syringe



Rotary Tray

ACQUITY UPLC H-Class System- SM-FTN

- SM-FTN Only
 - Designed for best combination of performance and ease of use
 - One injection mode
 - Just select the injection volume
 - Less stringent requirements for wash solvents
 - Needlewash solvent washes outside of needle only
 - Purge solvent does not contact sample



ACQUITY I-Class System

CH-A
High Temperature Column Heater
Active Pre-Heating

BSM 18K
Binary Solvent Manager



Detectors
TUV, PDA eλPDA, FLR, ELSD
Low Dispersion Flow Cell Available

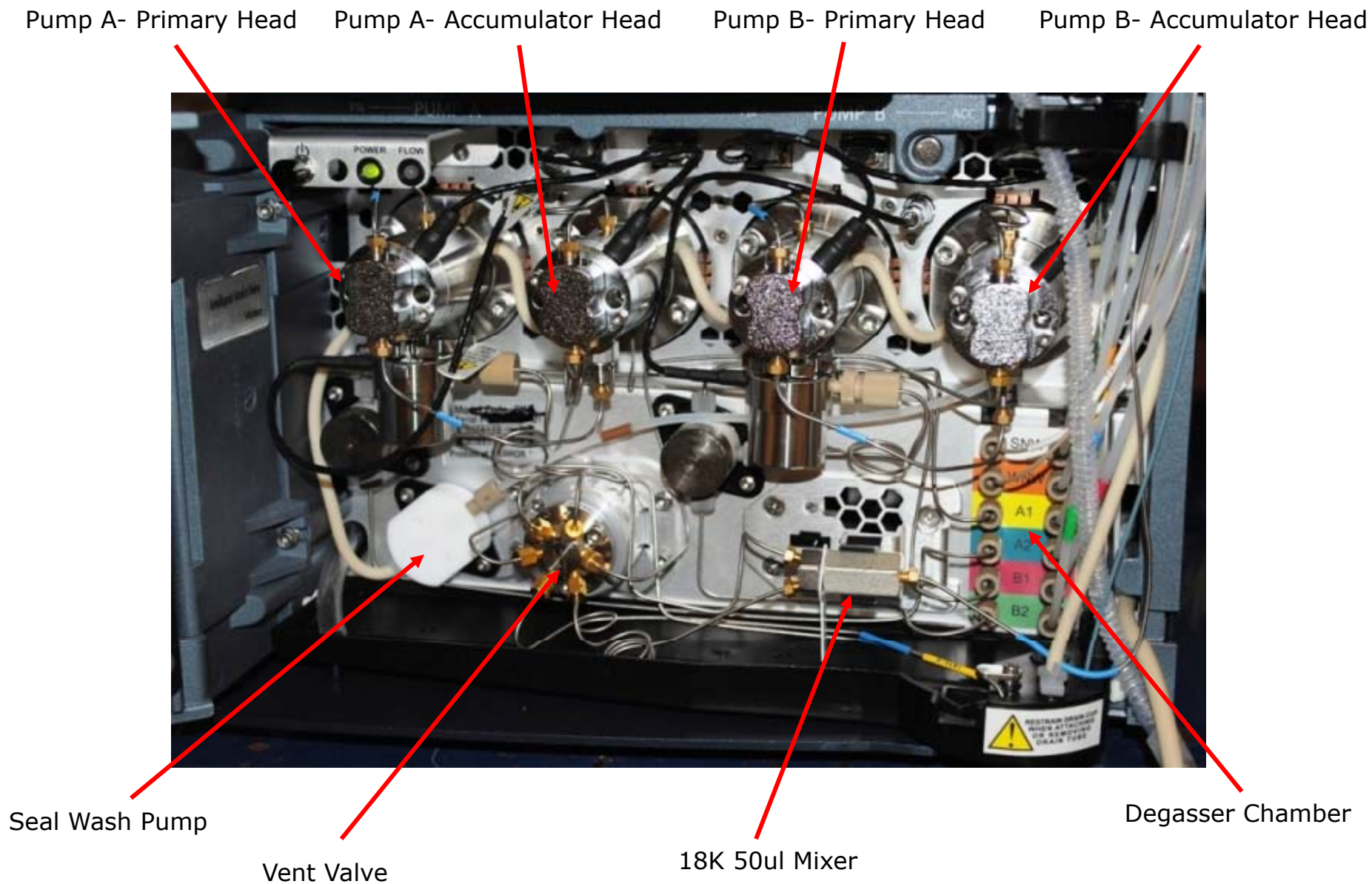
SM-FTN or SM-FL
Sample Manager-Flow Through Needle
Sample Manager- Fixed Loop

ACQUITY UPLC I-Class System

- Binary Solvent Manager (18K PSI Capable)
- Sample Manager (2 options)
 - Fixed-Loop (FL) Sample Manager
 - Flow-Through-Needle (FTN) Sample Manager
- Column Management (2 options)
 - Single Column Heater
 - Dual Column Manager
 - Max 2 columns
 - Optional 2D Technology feature
- Detection
 - MS
 - TUV or PDA only – new lower dispersion flow cells
- New ACQUITY UPLC Systems Driver Pack (June 2011 DP)
- Existing ACQUITY UPLC Chemistries



I-Class BSM Fluidics

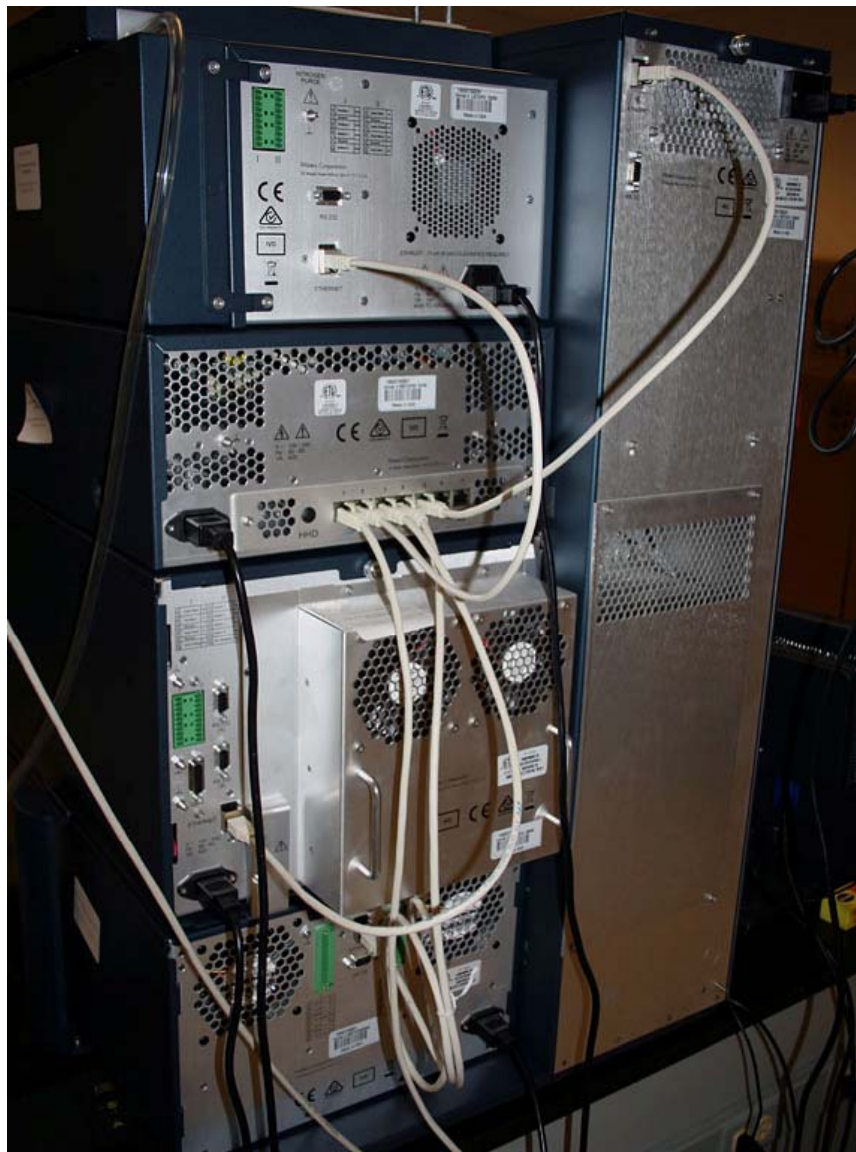


ACQUITY UPLC I-Class System Sample Manager

- FTN or FL Configuration
 - Lower dispersion injector components
 - 18K psi capable
 - Select based on application needs and laboratory work flow

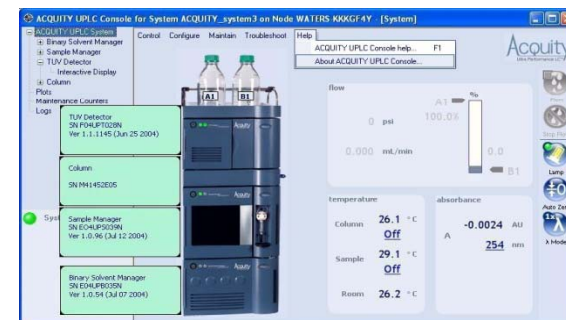


All Modules are Ethernet Controlled

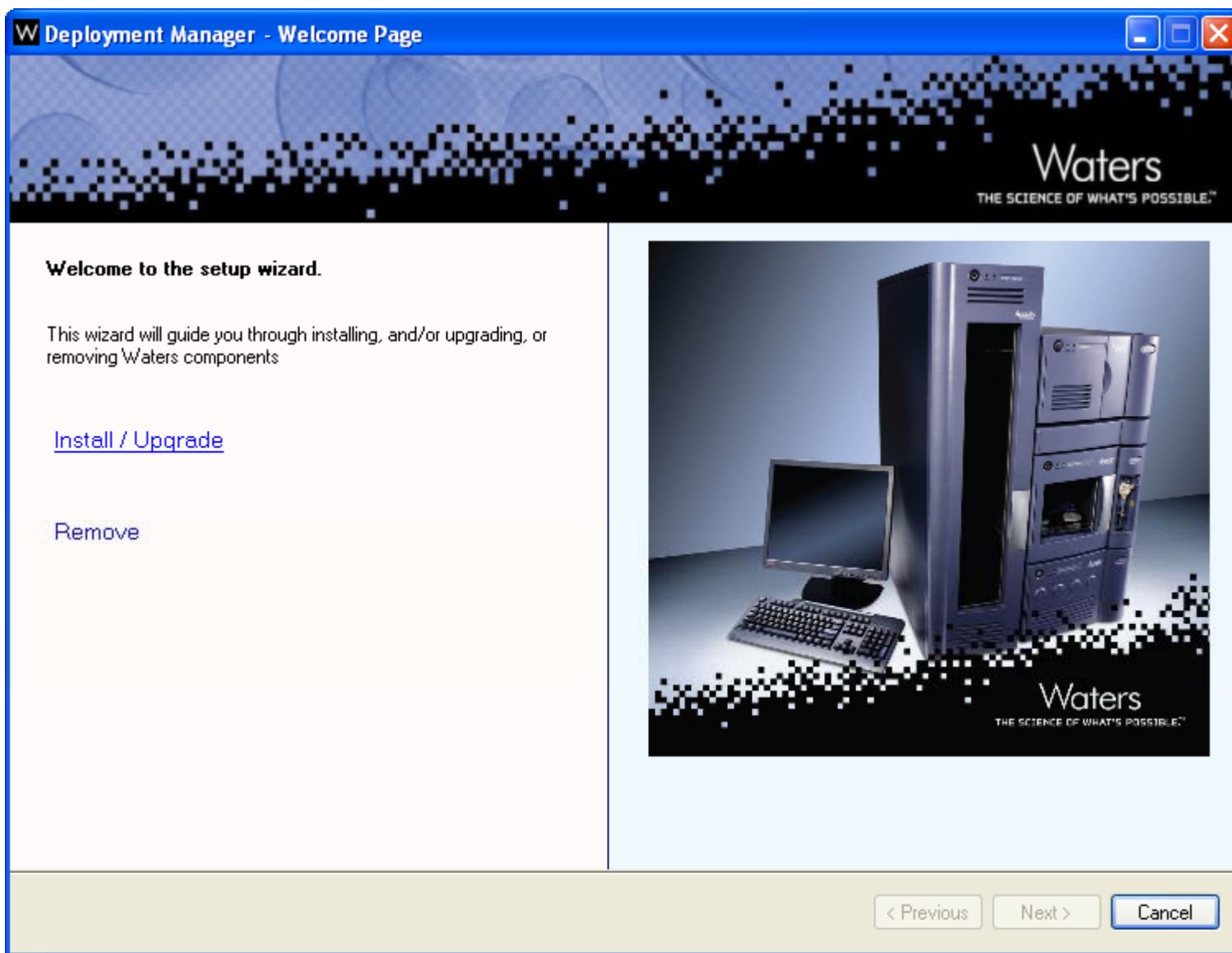


ACQUITY UPLC System - ACQUITY Console / System Driver

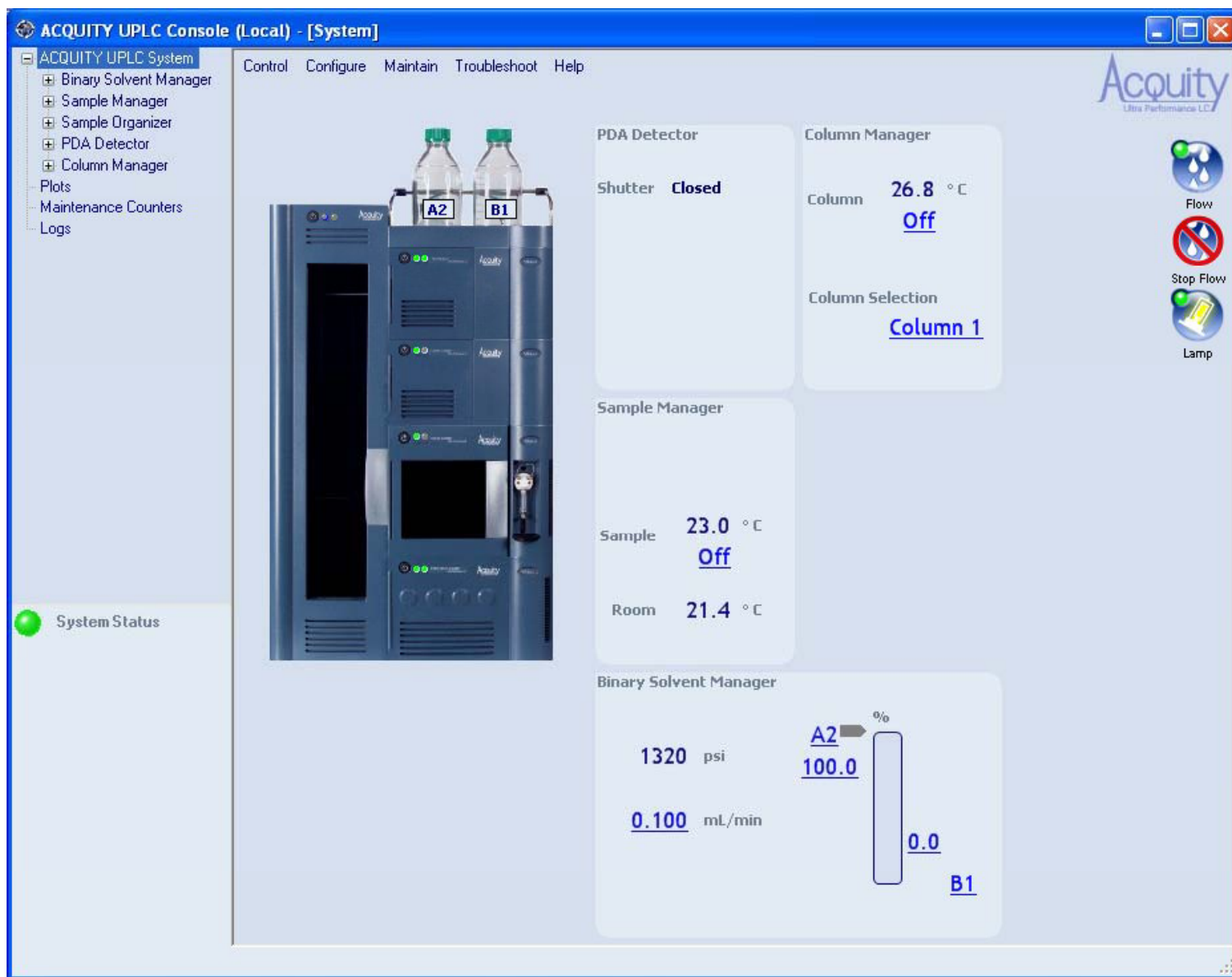
- Plug and Play open architecture
 - Software and firmware packaged together
 - Simple upgrade strategy
- Instrument Control Software
 - Separate portable application folds into:
 - MassLynx
 - Empower
 - Standalone
 - Other vendor's software
- Minimizes learning curve
 - Simple diagnostics - graphical
 - Single On-line Help
 - Connection InSight™ Support



Instrument Driver Install Menu



ACQUITY UPLC Console



ACQUITY Method Editor for Empower

Untitled in UV_PQ_Project as System/Administrator - Instrument Method Editor

File Edit View Help

Acquity UPLC Binary Solvent Manager ACQ-BSM Acquity UPLC Sample Manager ACQ-SM Acquity UPLC TUV Detector ACQ-TUV

Acquity Binary Solvent Manager

General | Data | Analog Out | Events

Solvents

A1 *Water ...
B1 *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	0.250	100.0	0.0	Initial
2					
3					
4					
5					
6					

Comment: ...

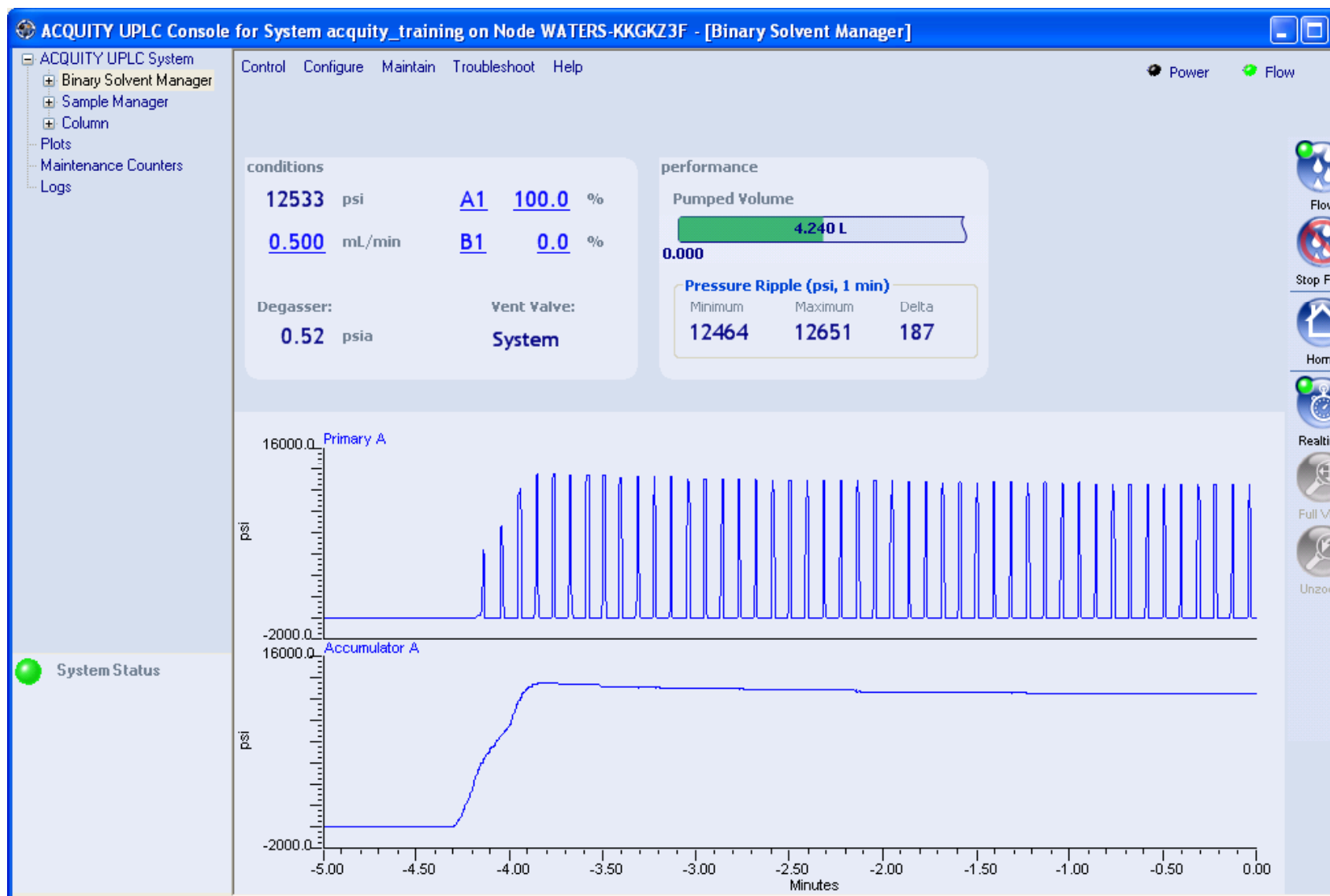
Ready

ACQUITY UPLC System - Solvent Delivery

- 15 K psi performance
 - Accurate and precise flow rates
 - Accurate and precise composition
 - Low delay volume for gradient applications
 - <120 μ L System Volume
 - Long term reliability



Single Pump Pressure Example



BSM Instrument Method

Modify ACQUITY Binary Solvent Manager Instrument Method

Acquity Binary Solvent Manager
Ultra Performance LC

Run Time: 1.00 min

General | Data | Analog Out | Events

Solvents

A1 *Water ...

B2 *Acetonitrile ...

Pressure Limits

Low: 100 psi

High: 15000 psi

Seal Wash: 5.0 min

Gradient

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.600	10.0	90.0	Initial
2	0.20	0.600	10.0	90.0	6
3	0.60	0.600	90.0	10.0	11
4	30.00	0.000	90.0	10.0	11
5					
6					
-					

Comment: ...

OK Cancel



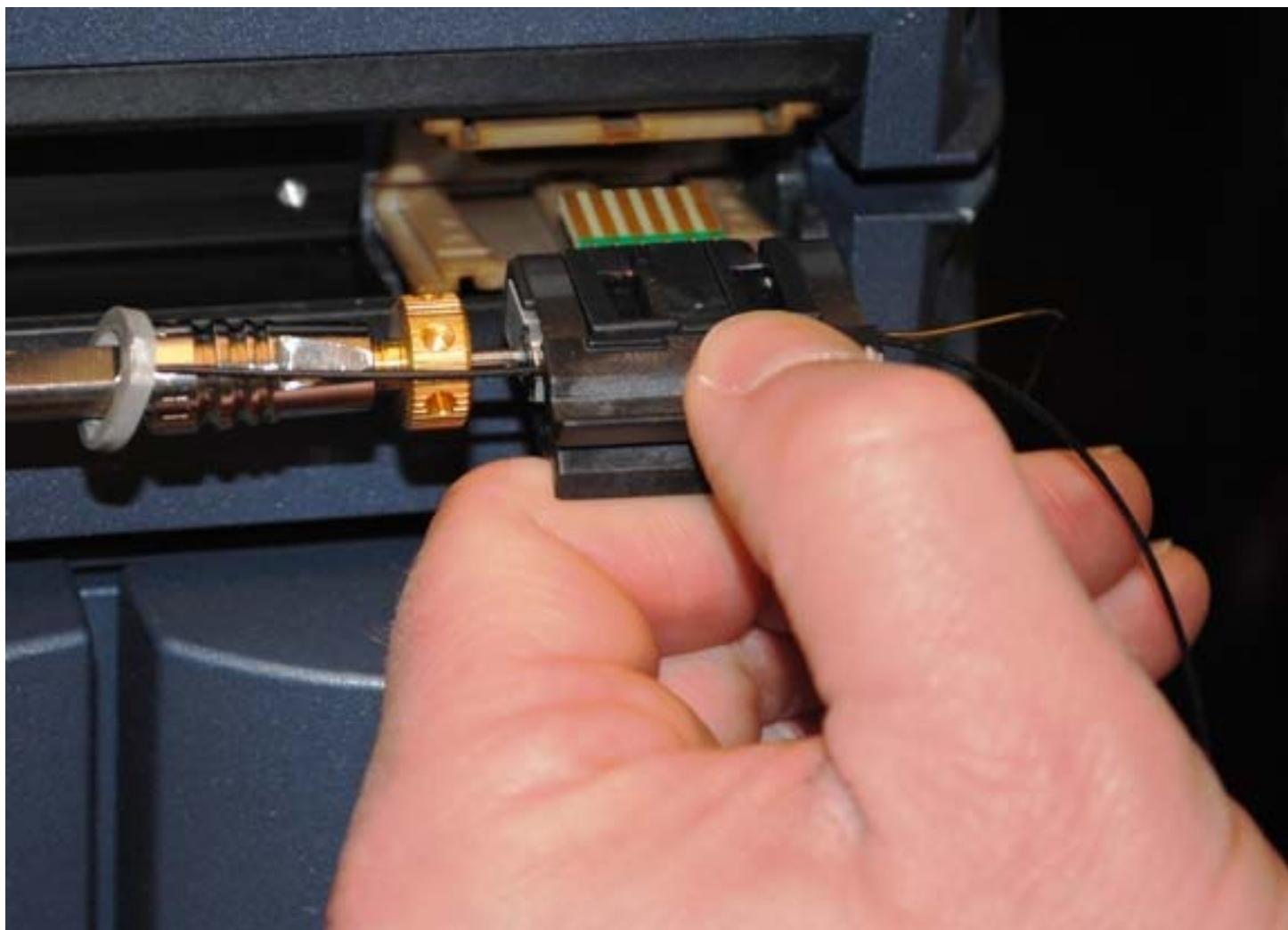
Column Heating and Cooling Options



CH-A



Installing the Active Pre-Heater



Finger-tight APH Fitting

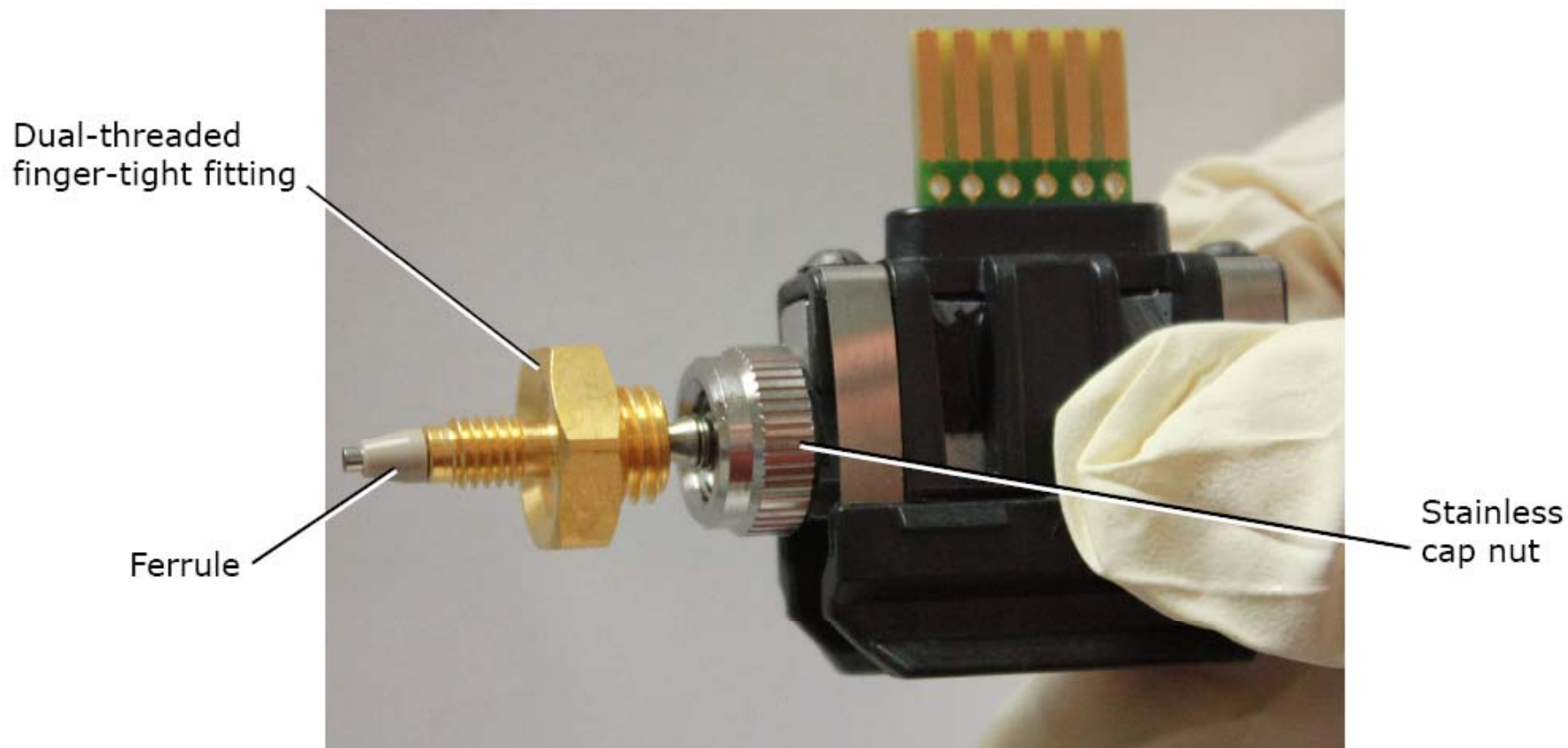
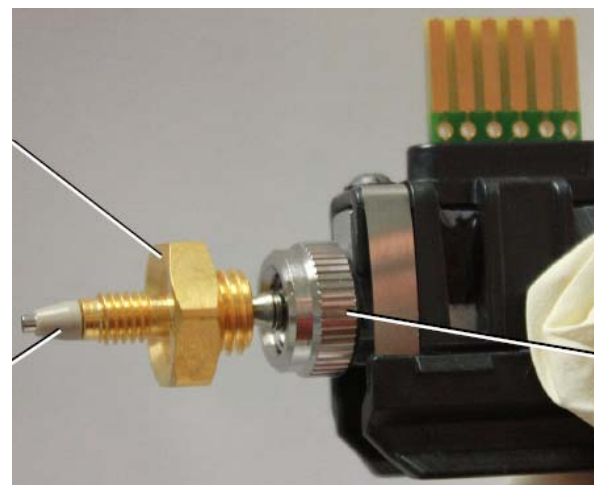


Figure 13 - Finger-tight APH fitting

Finger-tight APH Fitting Procedure

- Remove the APH assembly from the column compartment.
- Loosen stainless-steel cap nut from the finger-tight fitting.
- Insert the stainless-steel tubing with ferrule and fitting into the column.
 - Make sure the stainless-steel tubing protrudes beyond the ferrule.
- Seat the ferrule by securely finger-tightening the fitting into the column.
 - Do not engage the stainless-steel cap nut when seating the ferrule.
- Complete the connection by finger-tightening the stainless-steel cap nut onto the fitting.



CM-A (2 or 4-Columns)



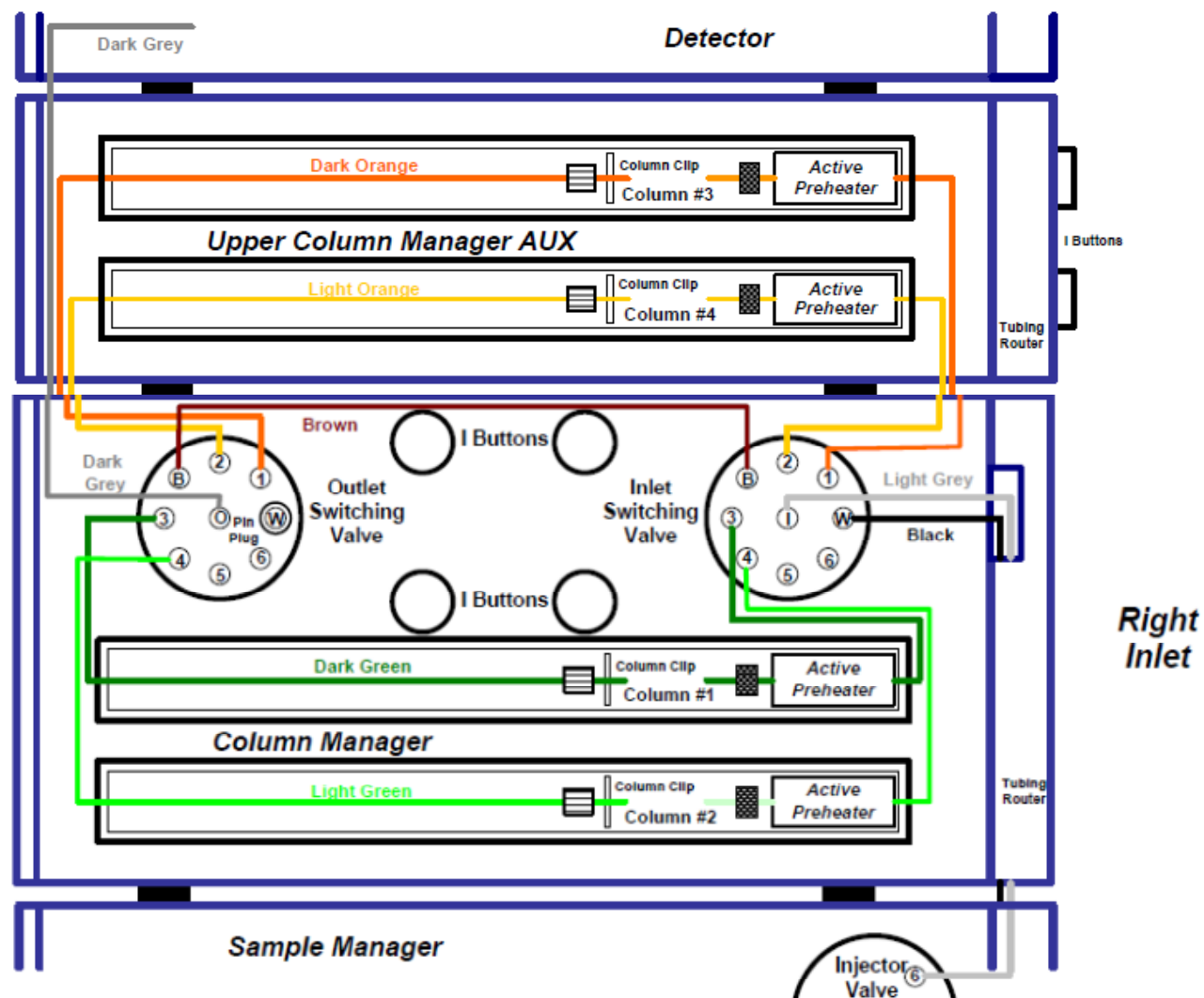
- CM-A
 - 2 x 150mm, or, 4 x 50mm* columns
 - 2 independent heat/cool zones
 - 2, 9-port switching valves
 - Movable active pre-heaters (APH) allow for either left or right inlet plumbing of the same module

CM-A and CM-Aux (4-Columns)

- CM-A and CM-Aux
 - 4 x 150mm columns
 - 4 independent heat/cool zones
 - Active pre-heaters (APH) as standard in all zones

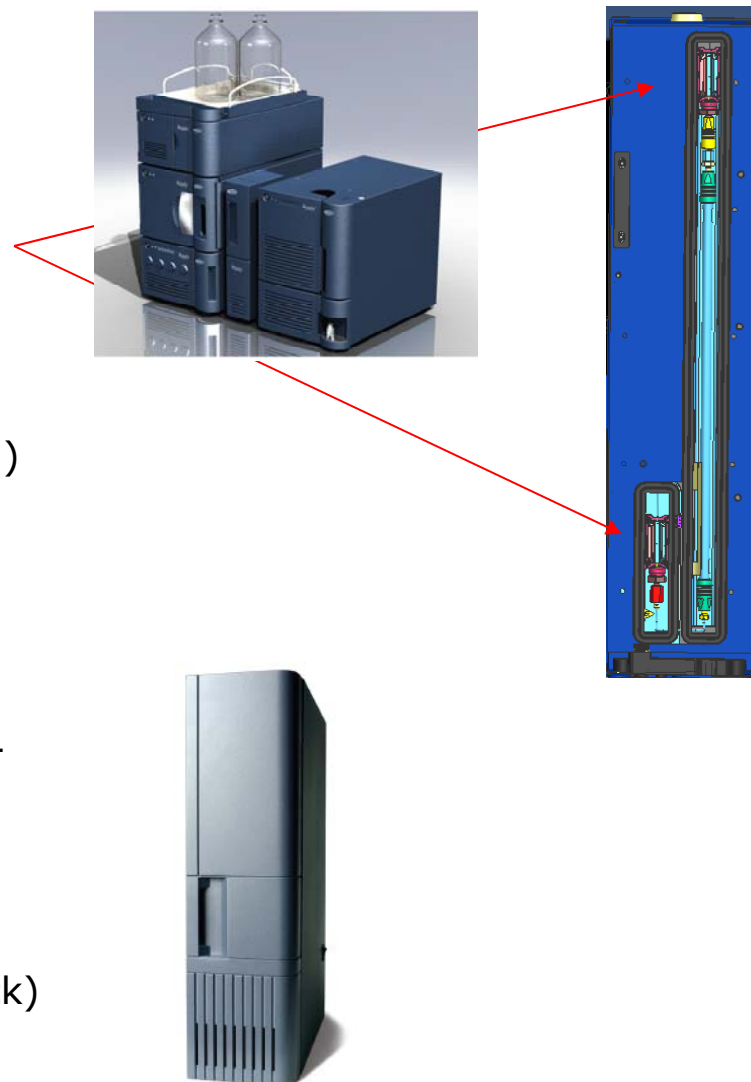


4 x 150mm Configuration



H-Class 30cm Column Compartments

- New 30cm Column Heater (CH-30A) - 176015046
 - Single column heater
 - Heats to 90°C
 - Supports up to 4.6mm ID
 - 2 APH locations
 - Enables optimum tubing configuration for both HPLC and UPLC applications (comes standard with HPLC length APH)
 - Requires v. 1.45 ICS or later
- Existing ACQUITY 30cm CHC - 186015011
 - For HPLC legacy support for >4.6mm ID columns
 - Requires H-Class compatibility kit (205000761)
 - Requires v. 1.46 ICS (June 2010 Driver Pack)





Common Platform Sample Organizer

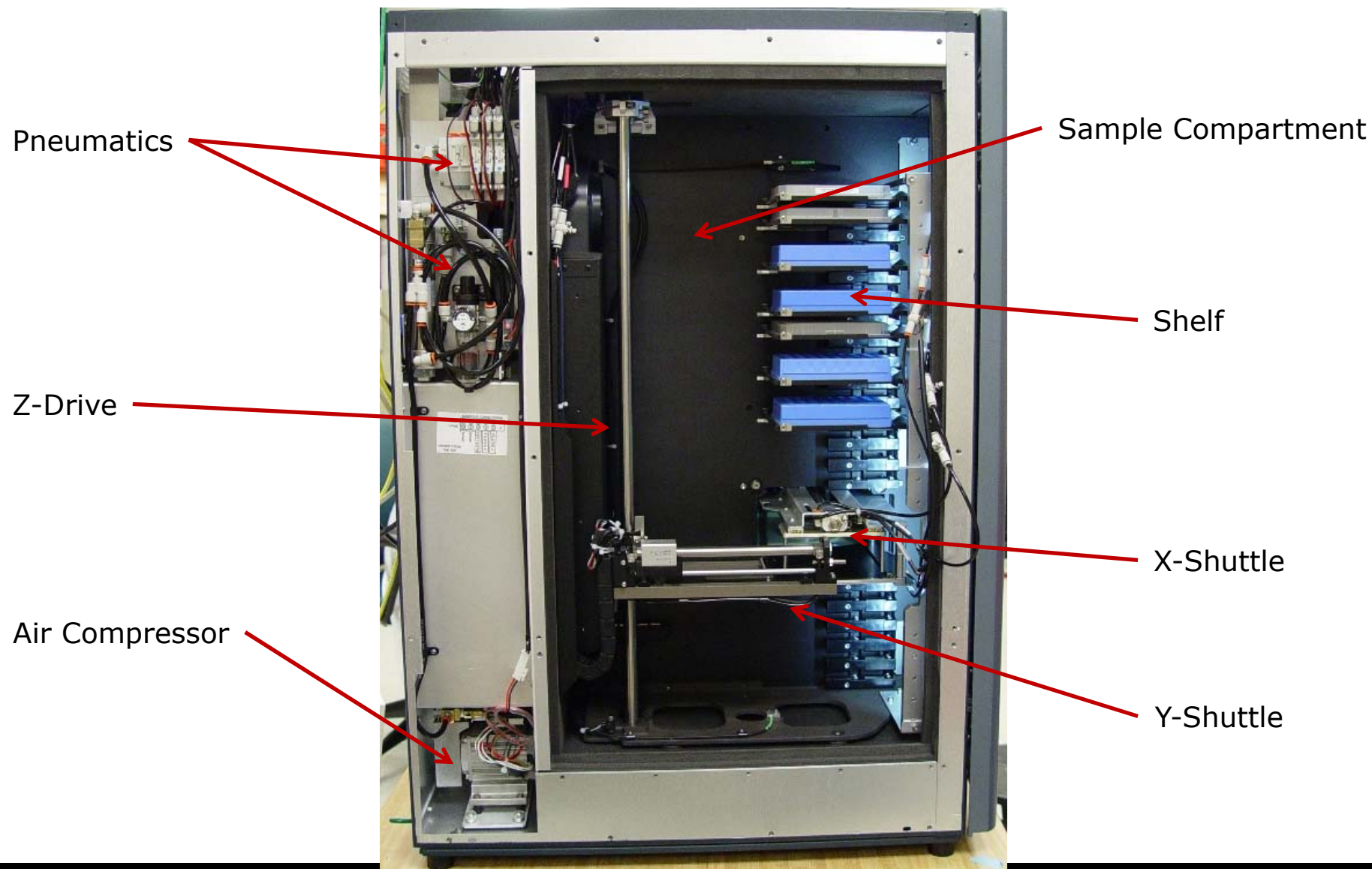


ACQUITY UPLC System - Optional Sample Organizer

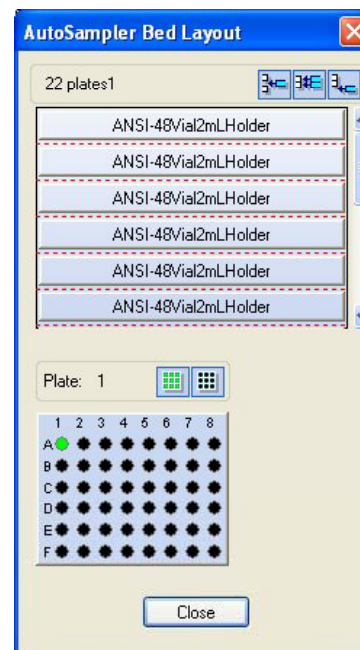
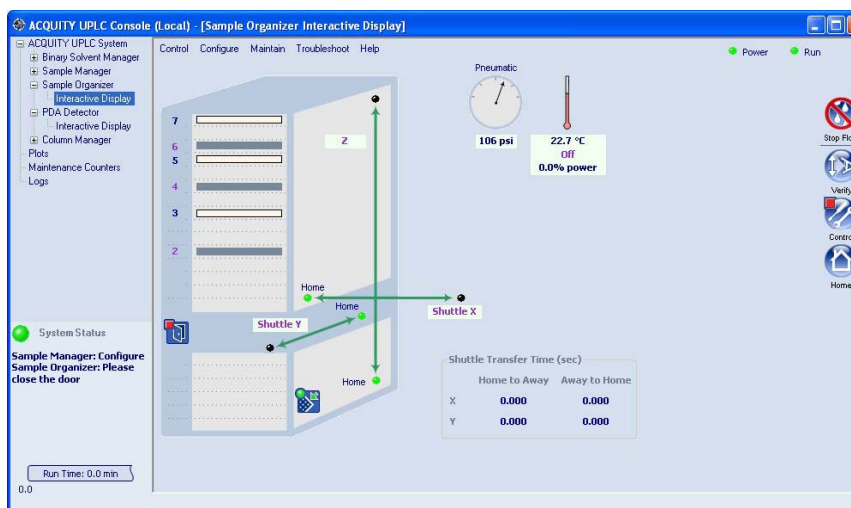
- Offers flexibility on sample format
 - 22 thin (microtitre) plates (*8,448 samples max*)
 - 15 medium height plates
 - 8 full height (vial) plates (*384 x 2mL vials*)
 - Temperature controlled
 - Programmable Access any sample location
 - Complements Column Manager
 - Run diverse samples, with diverse methodologies
 - maintains high sample throughput by accessing different chemistries



Major Functional Assemblies



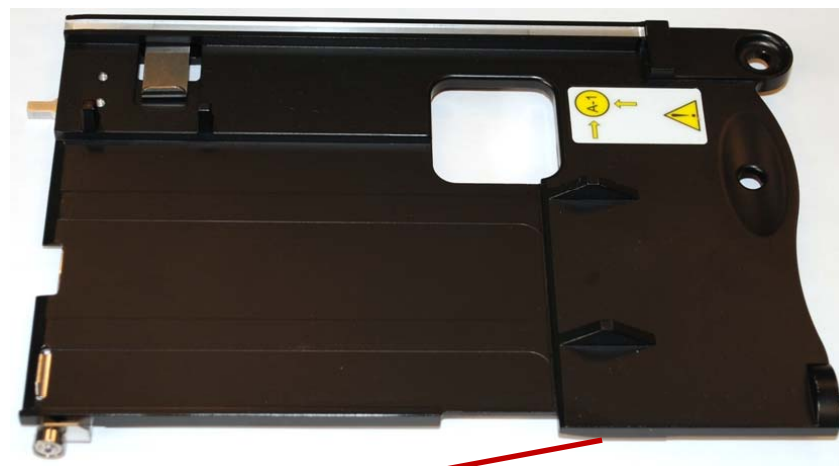
Sample Organizer



Shelf Stability Bracket

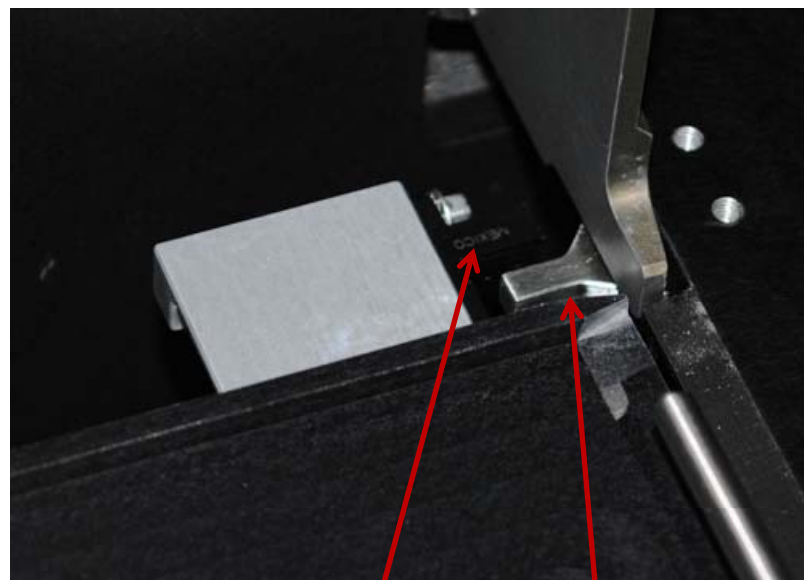
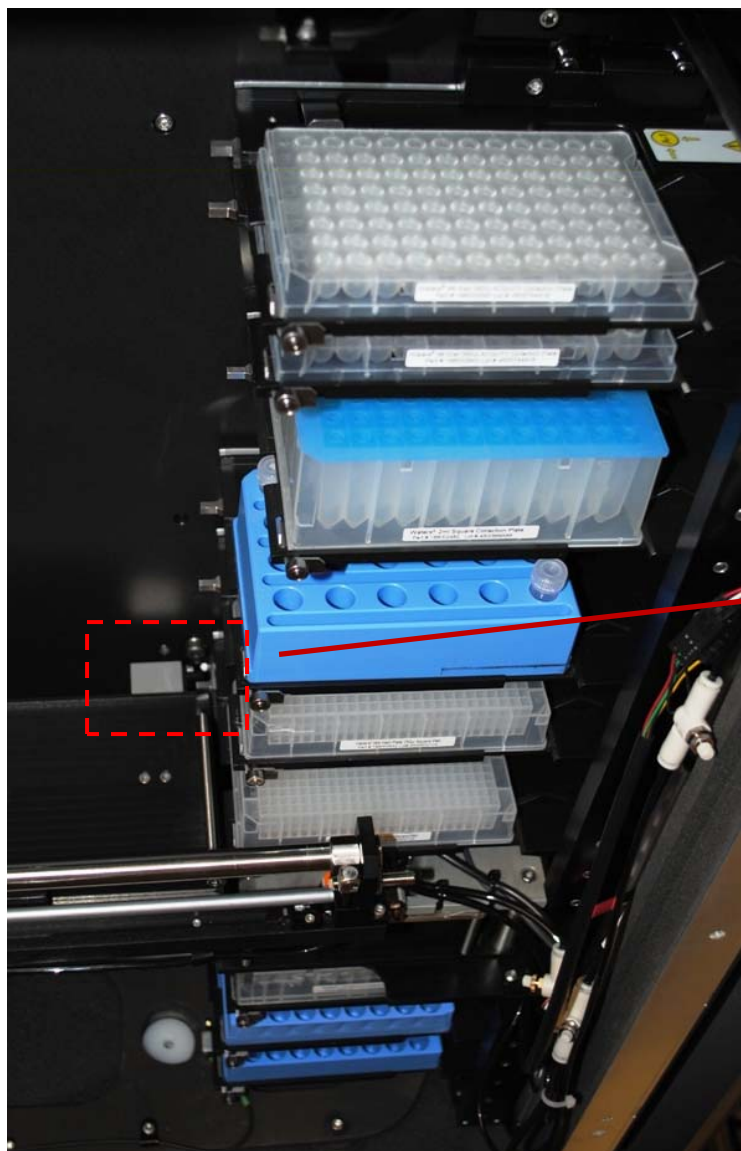


Shelf Stability Bracket



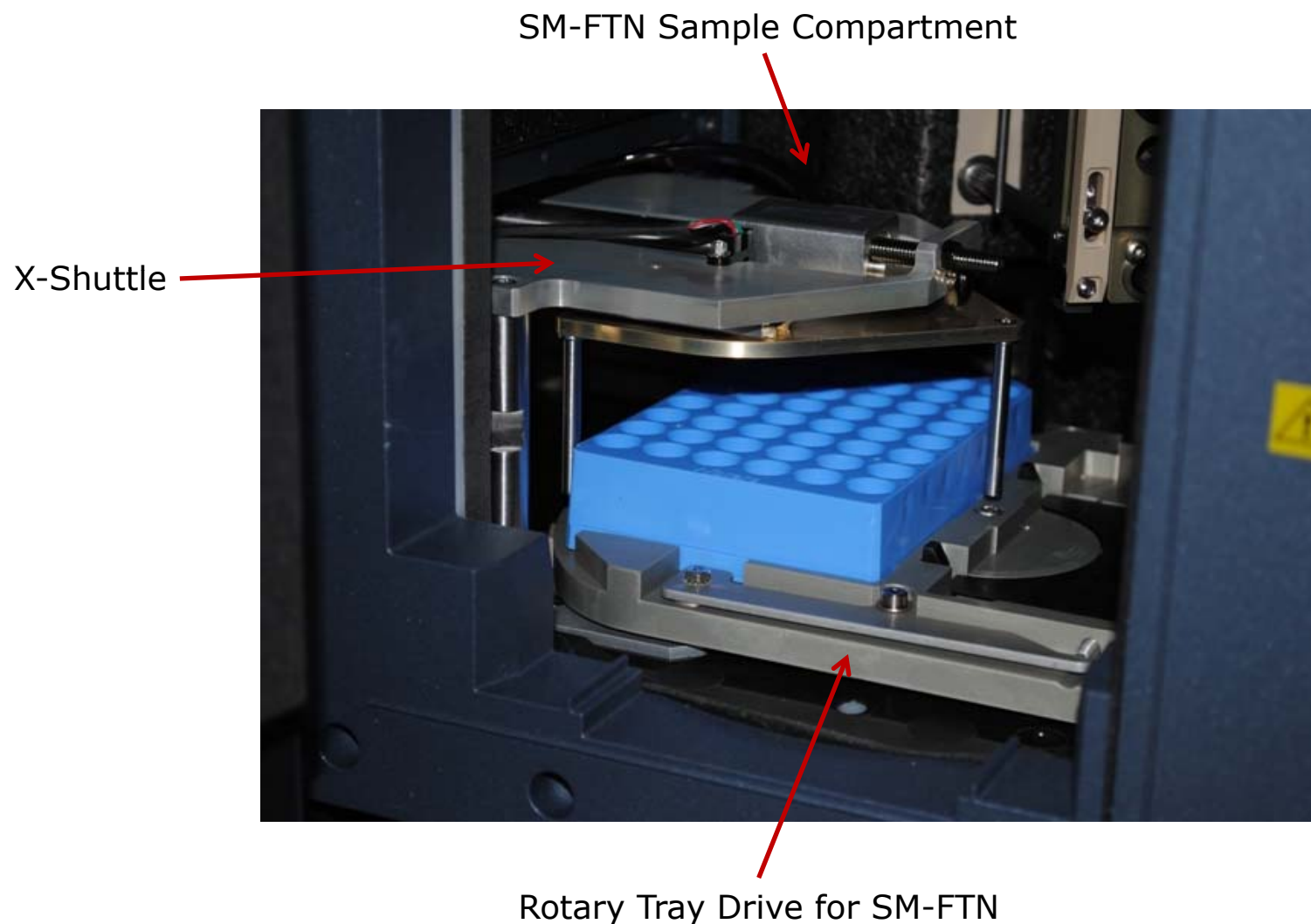
New wider Shelf

Shelf Position Sensor and Flag

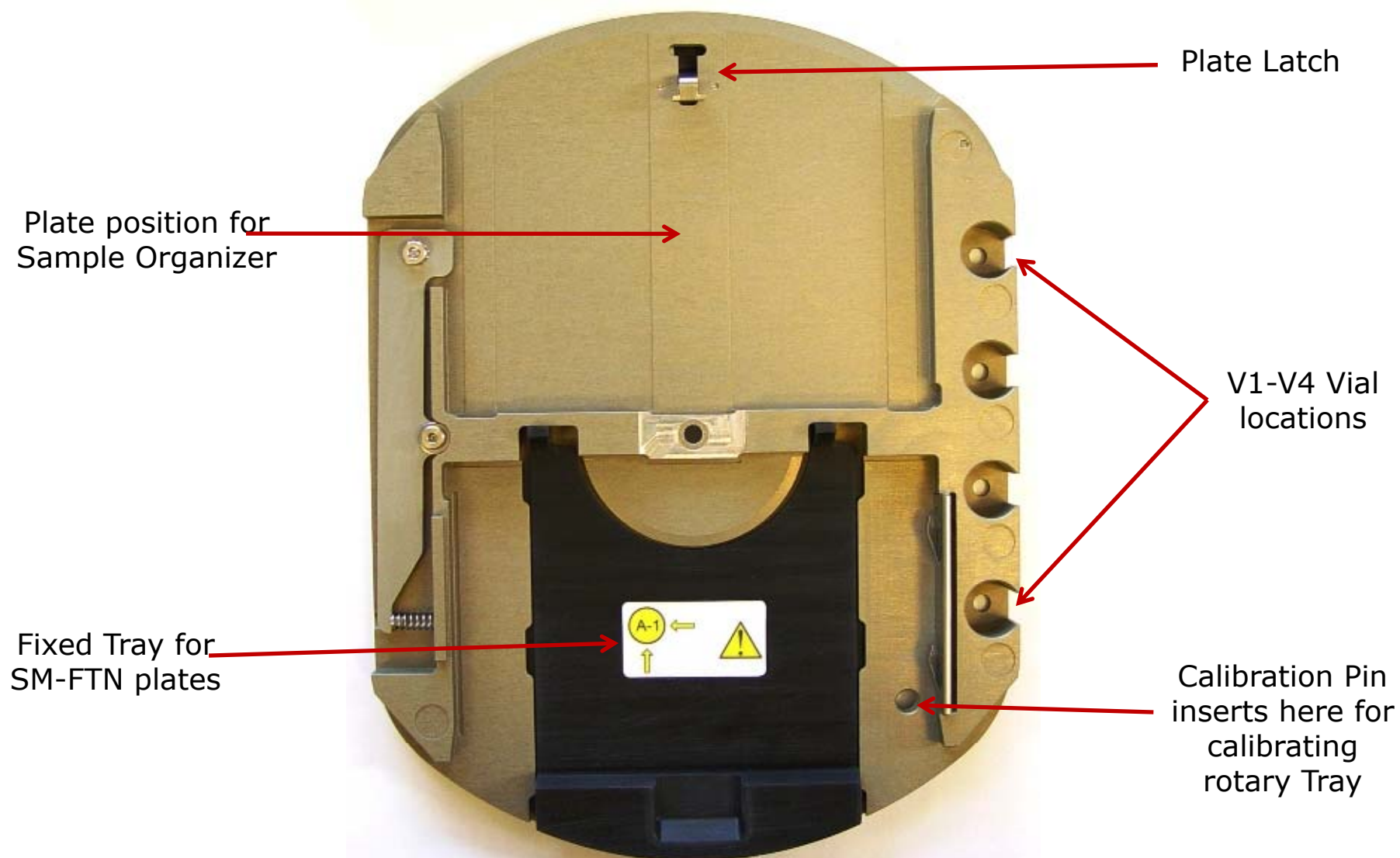


Shelf Sensor and Flag

X-Shuttle Assembly Installed



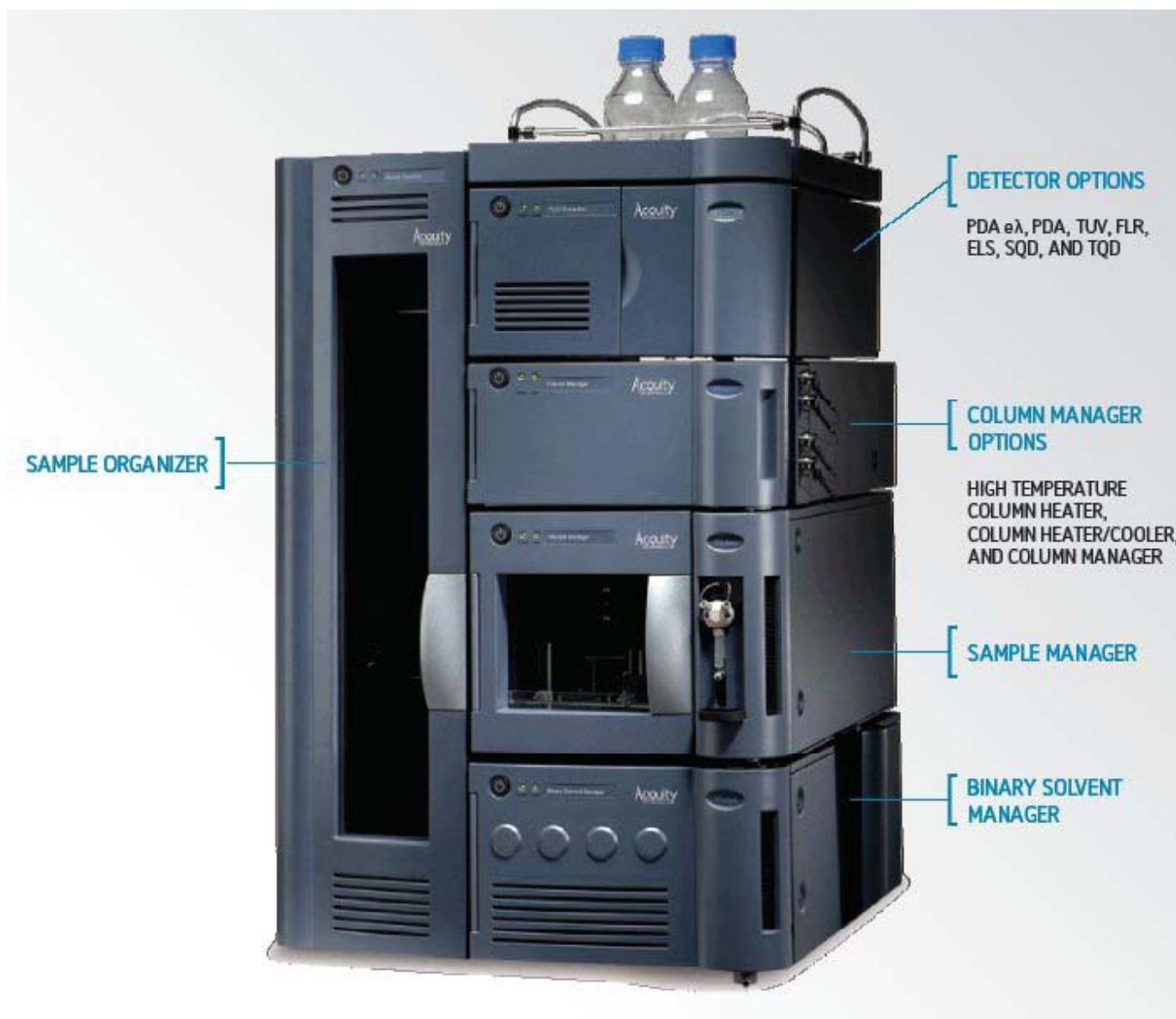
Rotary Tray Drive for Sample Organizer



ACQUITY Classic System Overview

ACQUITY UPLC® Service Training Introduction

ACQUITY UPLC Classic System



ACQUITY UPLC Classic System



❖ Introduced in 2004

❖ Binary Solvent Manager

- ❖ Binary high pressure blending w/ 4 solvent capacity
- ❖ Low dispersion design, UPLC pressure capable

❖ Sample Manager

- ❖ Fast variable volume injector
- ❖ Dual wash - low carryover
- ❖ Plates and/or vials (4-40°C)

❖ Sample Organizer

- ❖ Expands capacity (22/15/8 Plate/Vial Trays)
- ❖ Shuttle plate feeder, heated/chilled

❖ Column Manager (3 Choices)

- ❖ Single column: ambient to 90°C
- ❖ Column heater/chiller: 10 to 90°C
- ❖ Column heater/chiller with 4 column switching
- ❖ Column heater/chiller for legacy methods

❖ ACQUITY UPLC Detectors

- ❖ Tunable UV, PDA, Fluorescence, ELSD, MS and MS/MS
- ❖ Optimized for UPLC...
 - ❖ Ultra-low dispersion
 - ❖ UPLC compatible sampling rates (80 Hz)
 - ❖ Sensitivity

❖ Multiple Data Systems

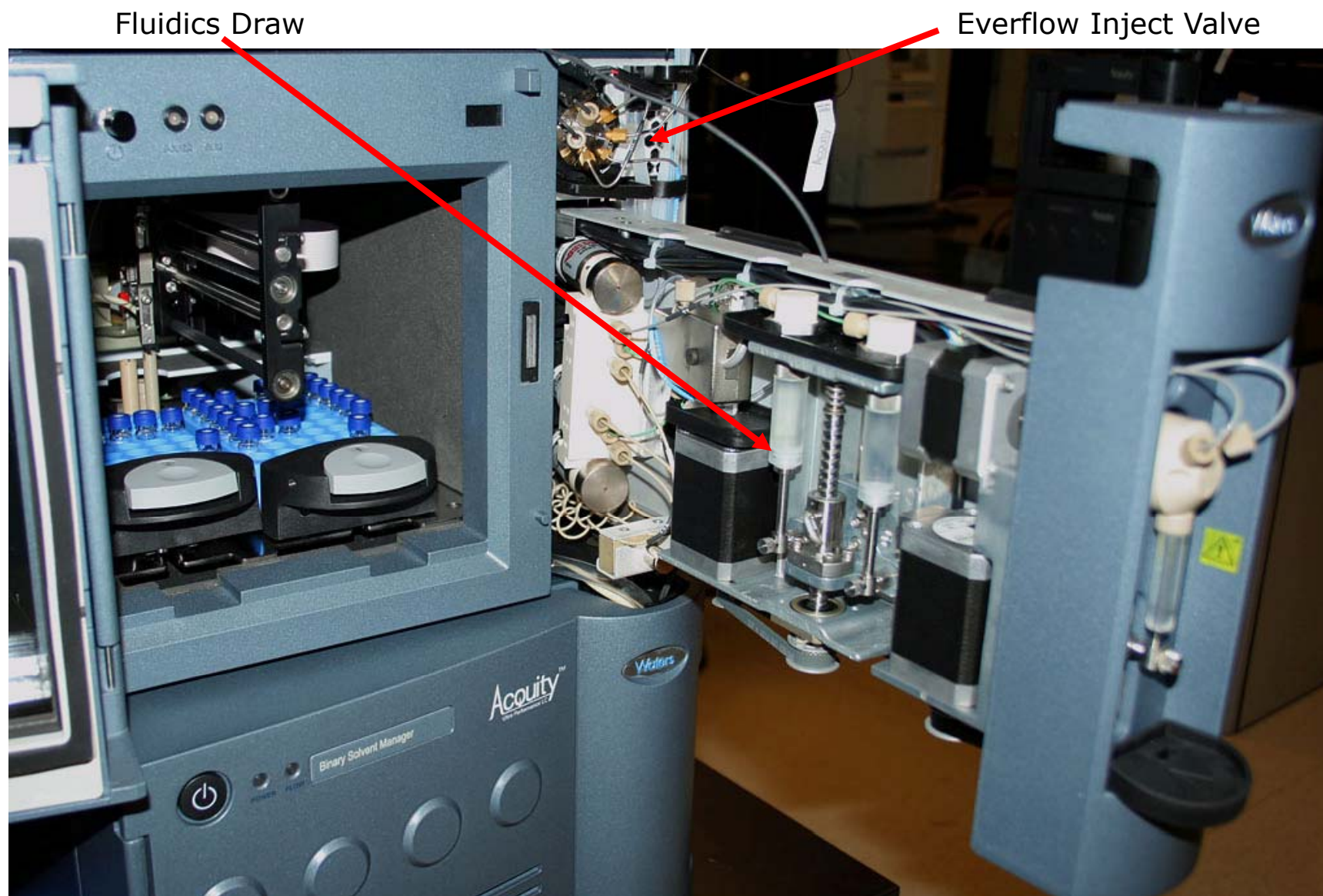
- ❖ Empower™, Empower 2 & Empower 3
- ❖ MassLynx™ 4.0 and 4.1
- ❖ Stand-Alone Control w/ Console
- ❖ Compatible with many of ABI/Sciex, ThermoFisher and Bruker Mass Spectrometers

ACQUITY UPLC System - Sample Management

- Fast cycle times
 - High speed injection sequence
- Smaller scale
 - Carryover <0.005%
 - Dual wash
 - 1 through 50 μL loops
- High pressure
 - Rugged valve switching design
 - Withstand high pressure actuation
 - No impact on column life
- XYZZ Sample transport
- Being replaced by new Common Platform SM



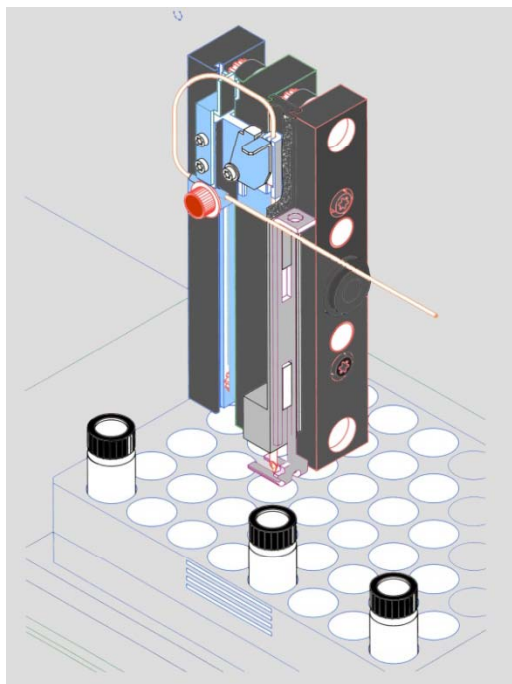
Sample Manager Fluidics- Loop Based Injector



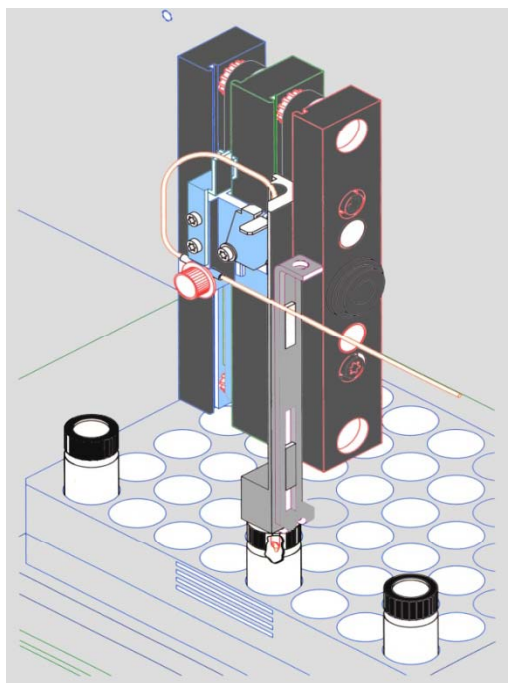
Supports Two ANSI STD Plates in XY Plane



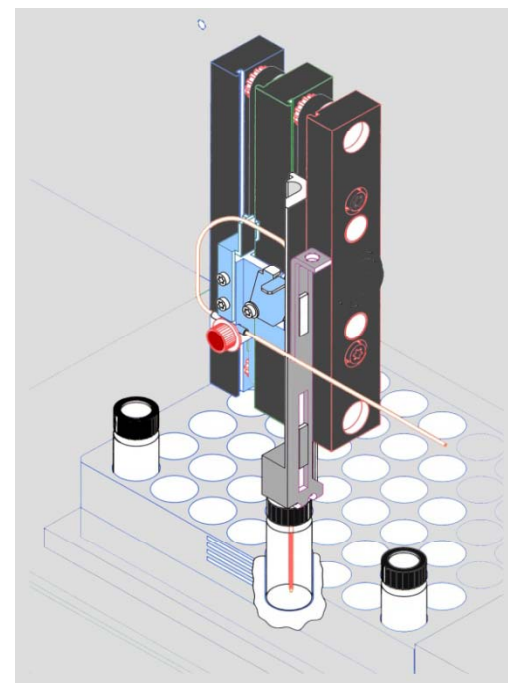
Needle-in-Needle Design



1. Locate sample vial



2. Puncture the cover

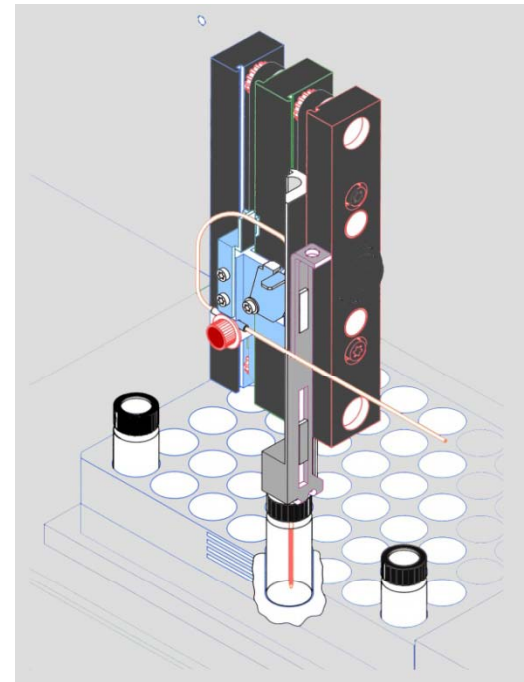


3. Lower sampling probe and aspirate sample

Needle-in-Needle Design

Advantages

- Virtually eliminates blockage of the sampling probe or injector by debris from the puncture of the cap of the sample vials
- Low dispersion in the sampling needle



Sample Manager Method Editor

Modify ACQUITY Sample Manager Instrument Method

Acquity Sample Manager
Ultra Performance LC

Run Time: 1.00 min

General | Data | Events | Column Manager

Sample Loop Option [Please Read!](#)

☐ Partial Loop
☒ Partial Loop With Needle Overfill
☐ Full Loop

Loop Offline: min
☐ Load Ahead

Wash Solvents

Weak: μL

Strong: μL

Temperature

Column: $^{\circ}\text{C}$ Alarm Band: ☐ $\pm$ $^{\circ}\text{C}$

Sample: $^{\circ}\text{C}$ ☒ $\pm$ $^{\circ}\text{C}$

Comment:

 Advanced...

OK Cancel

ACQUITY UPLC System

Sample Management – Instrument Method

- Full Loop
 - Using Pressure Assist injection technique
 - First choice (variable wastage & max cycle time)
- Partial Loop
 - Using Needle Overfill injection technique
 - Best recovery/accuracy
 - 15 μ L sample wastage & longer cycle time
 - Using Pressure Assist injection technique
 - Fastest
 - No sample wastage
- Other parameters
 - Loop Offline
 - Load Ahead
 - Variation of wash composition & duration



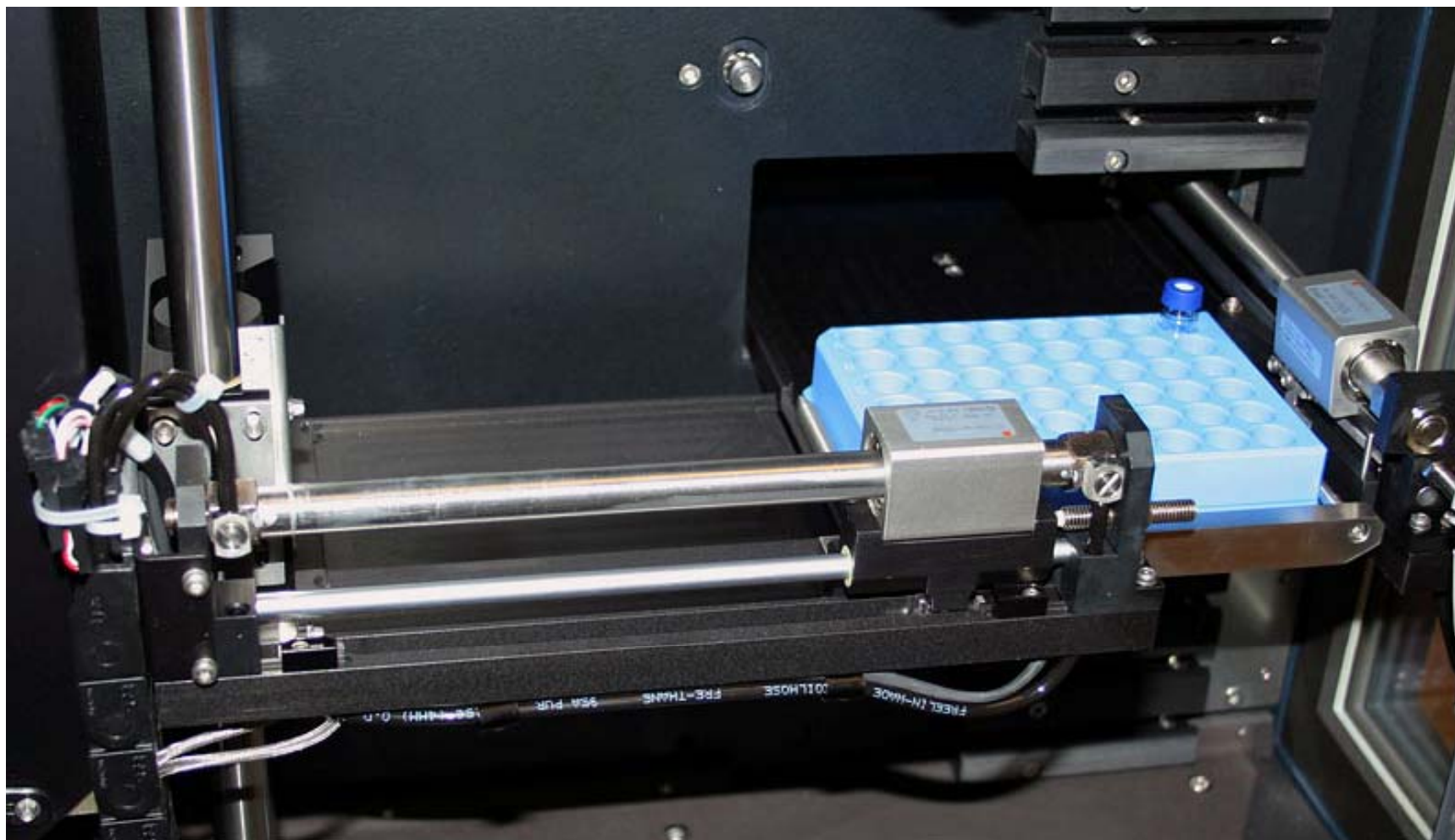
Classic Sample Organizer



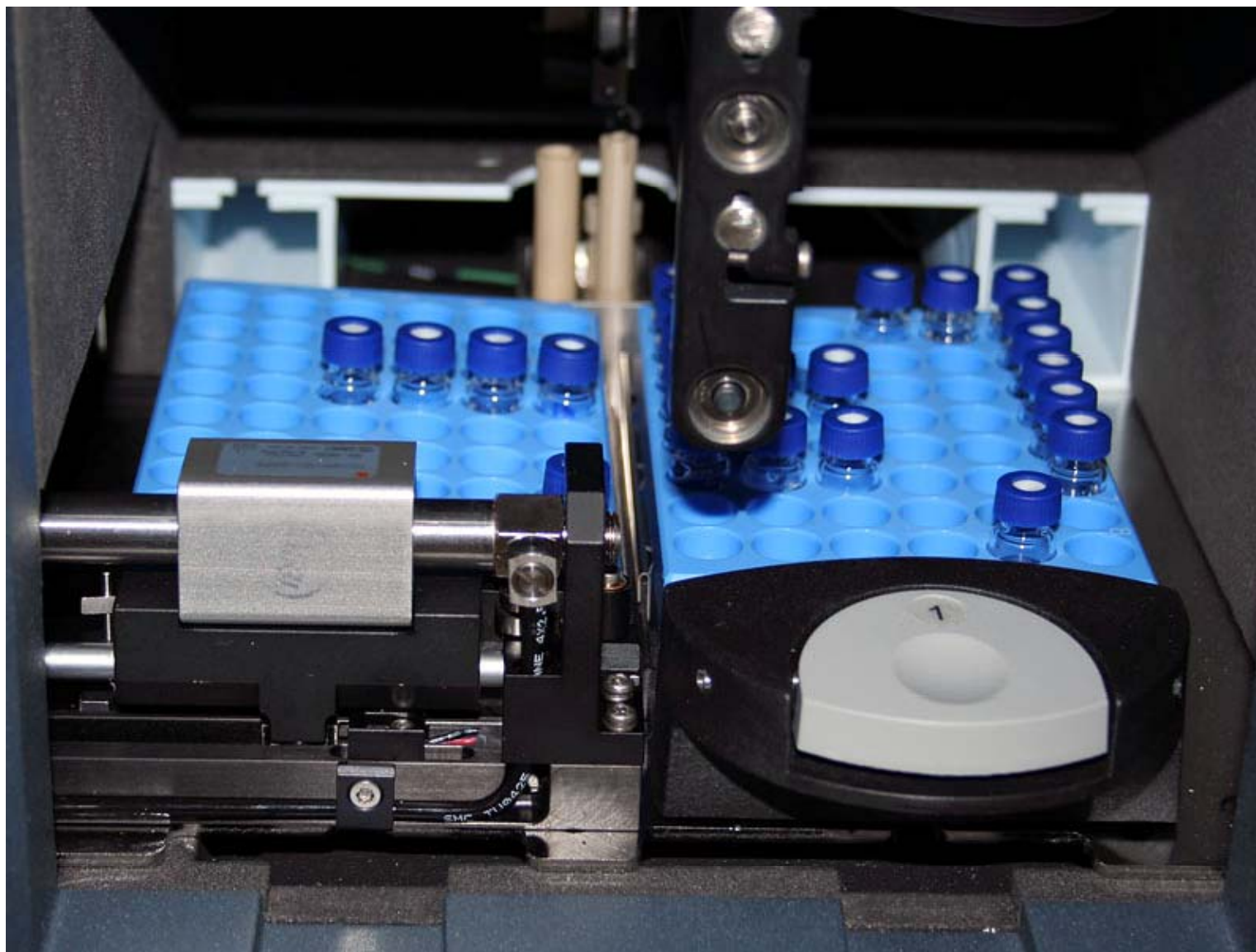
Sample Organizer - Open Side-View



Pneumatic - Driven Transport Shuttles



SM Configuration with Sample Organizer



ACQUITY UPLC System

3 Column Management Configurations

1. Sample Manager w/ Column Heater Module

- Heating only- Ambient to 90°C



2. Sample Manager w/ Column Heater/Cooler Module

- Heating and cooling- 10°C to 90°C



3. Sample Manager w/ Column Manager

- Heating and cooling 10°C to 90°C + 2 valves

- 4 column selection

- Bypass column and divert to waste



■ ACQUITY UPLC 30 cm Column Heater/Cooler

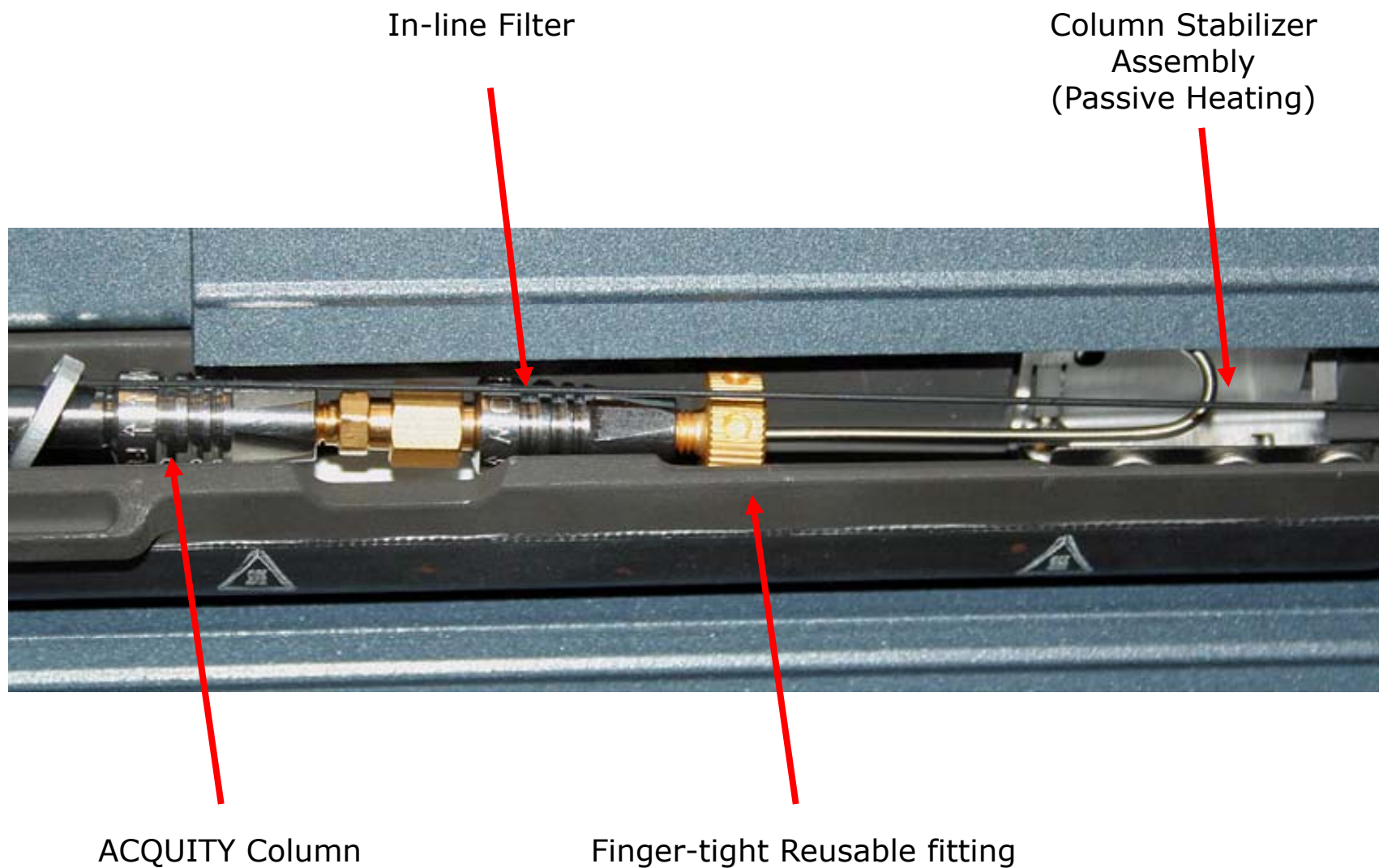
- Accessory with the three system option above



HT Column Heater with Passive Heating



Column Heater Compartment



Sample Manager Method Editor

Modify ACQUITY Sample Manager Instrument Method

Acquity Sample Manager
Ultra Performance LC

Run Time: 1.00 min

General | Data | Events | Column Manager

Sample Loop Option [Please Read!](#)

☐ Partial Loop
☒ Partial Loop With Needle Overfill
☐ Full Loop

Loop Offline: min
☐ Load Ahead

Wash Solvents

Weak: μL

Strong: μL

Temperature

Column: $^{\circ}\text{C}$ Alarm Band: ☐ $\pm$ $^{\circ}\text{C}$

Sample: $^{\circ}\text{C}$ ☒ $\pm$ $^{\circ}\text{C}$

Comment:

 Advanced...

OK Cancel

ACQUITY UPLC 30cm Column Heater / Cooler

To accommodate two 30cm HPLC Columns

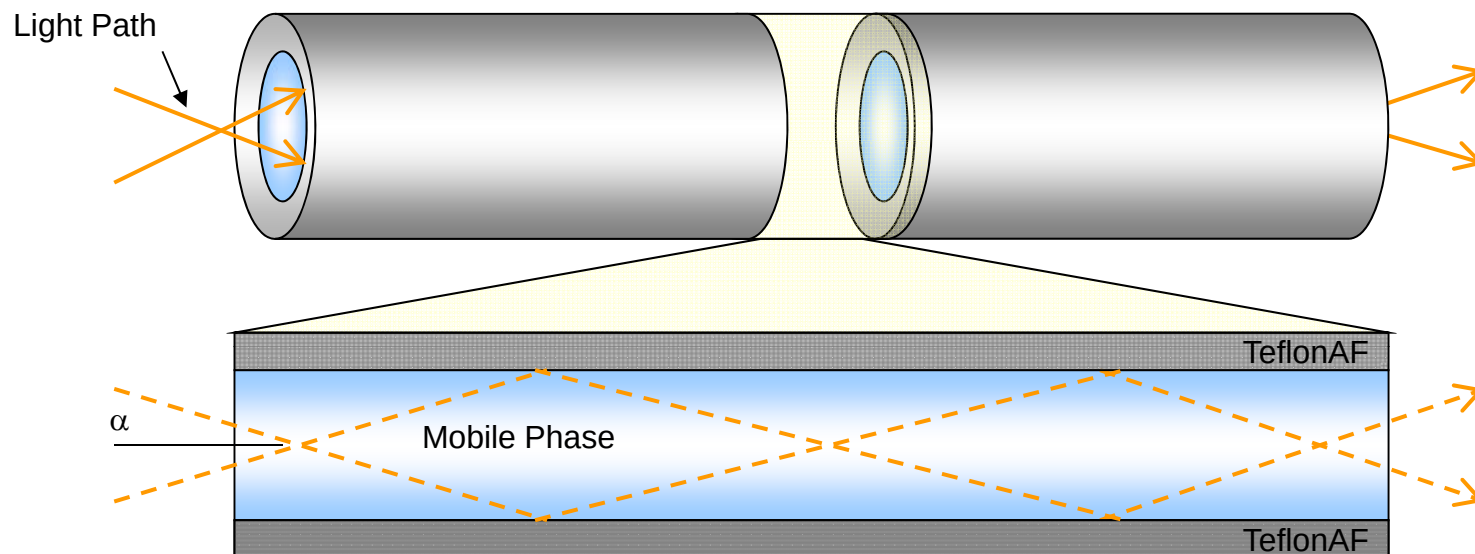


ACQUITY UPLC System - Detectors

- ACQUITY TUV, PDA and eλPDA
 - 10/25 mm path and 0.5/2.4 μ L volume
 - Sampling rate 80 Hz
- Wide wavelength range
 - TUV: 190 to 700 nm
 - PDA: 190 to 500 nm
 - e λ PDA: 190 to 800 nm
- Other detectors available today:
 - ELSD
 - FLR - flow cell <2 μ L
 - Waters SQD and TQD



Light Guided Flow Cells- Total Internal Reflection



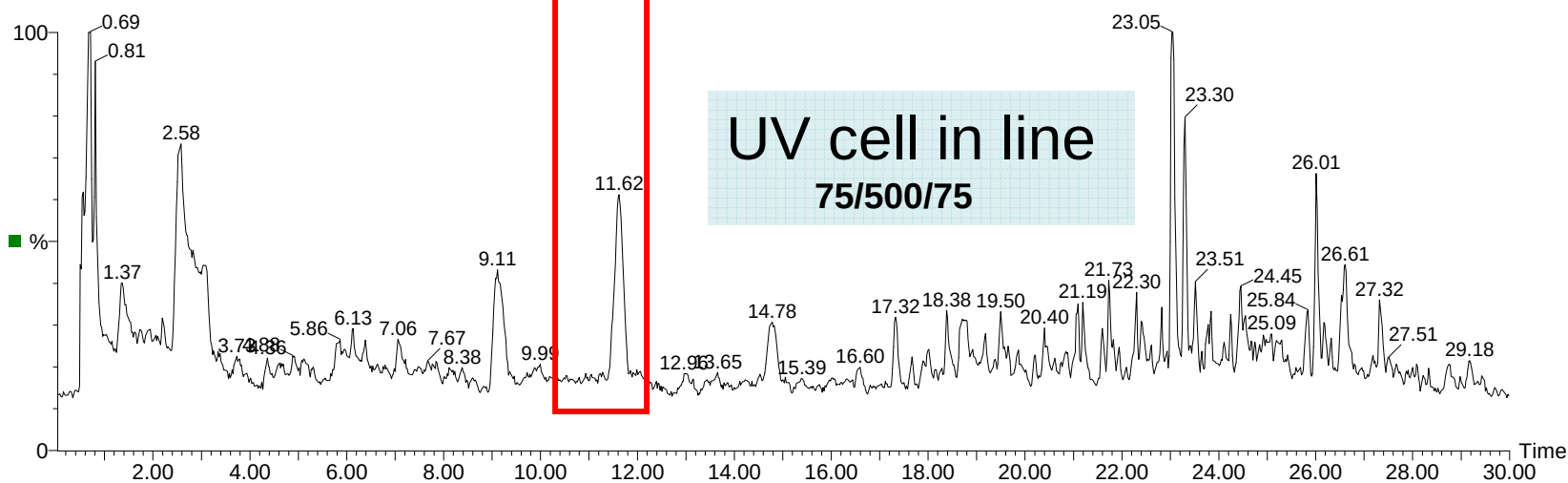
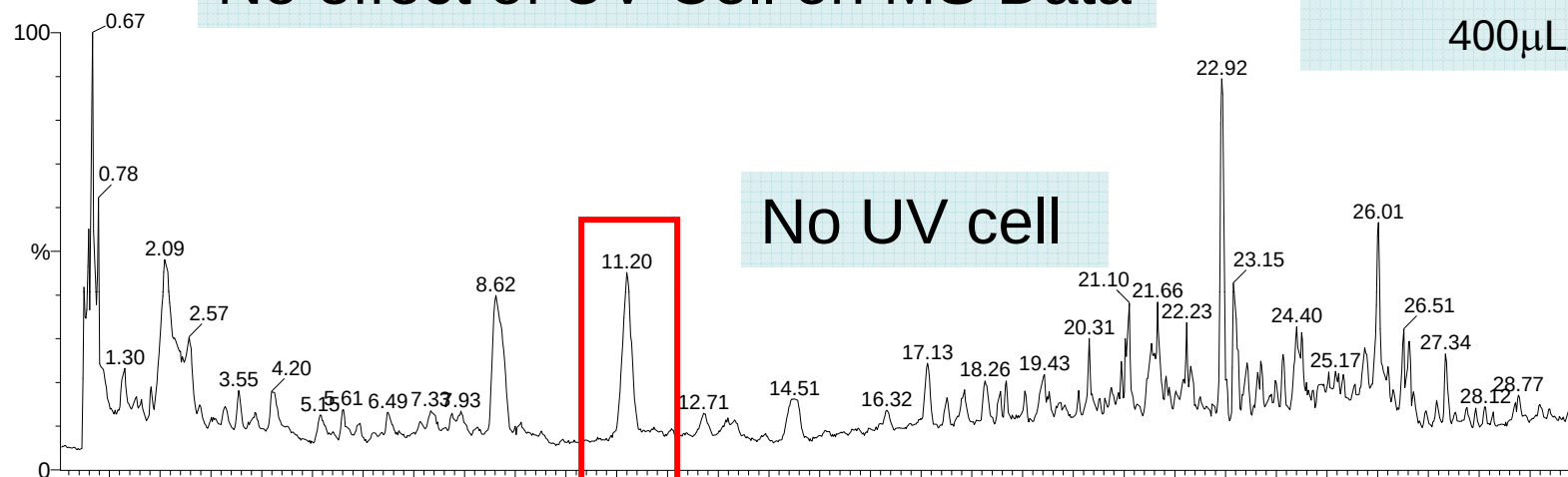
- Light rays entering the liquid core of the flow cell are internally reflected when they meet the Teflon AF boundary.
 - These rays are transmitted through the cell without loss, except for absorption by the sample
- Allows for good sensitivity while keeping flow cell volume (and consequently dispersion) to a minimum

ACQUITY UPLC System

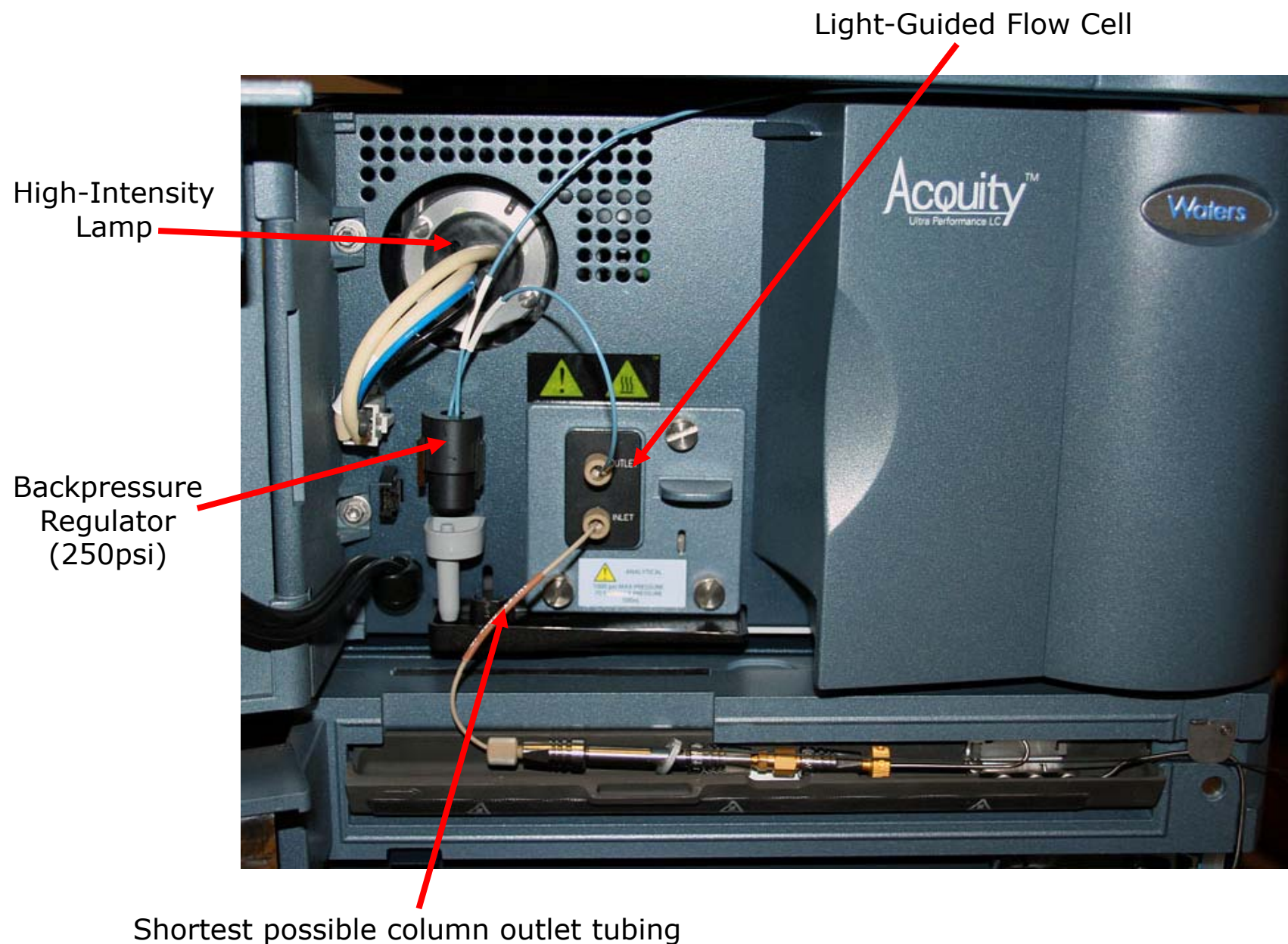
Low dispersion UPLC flow cells

No effect of UV Cell on MS Data

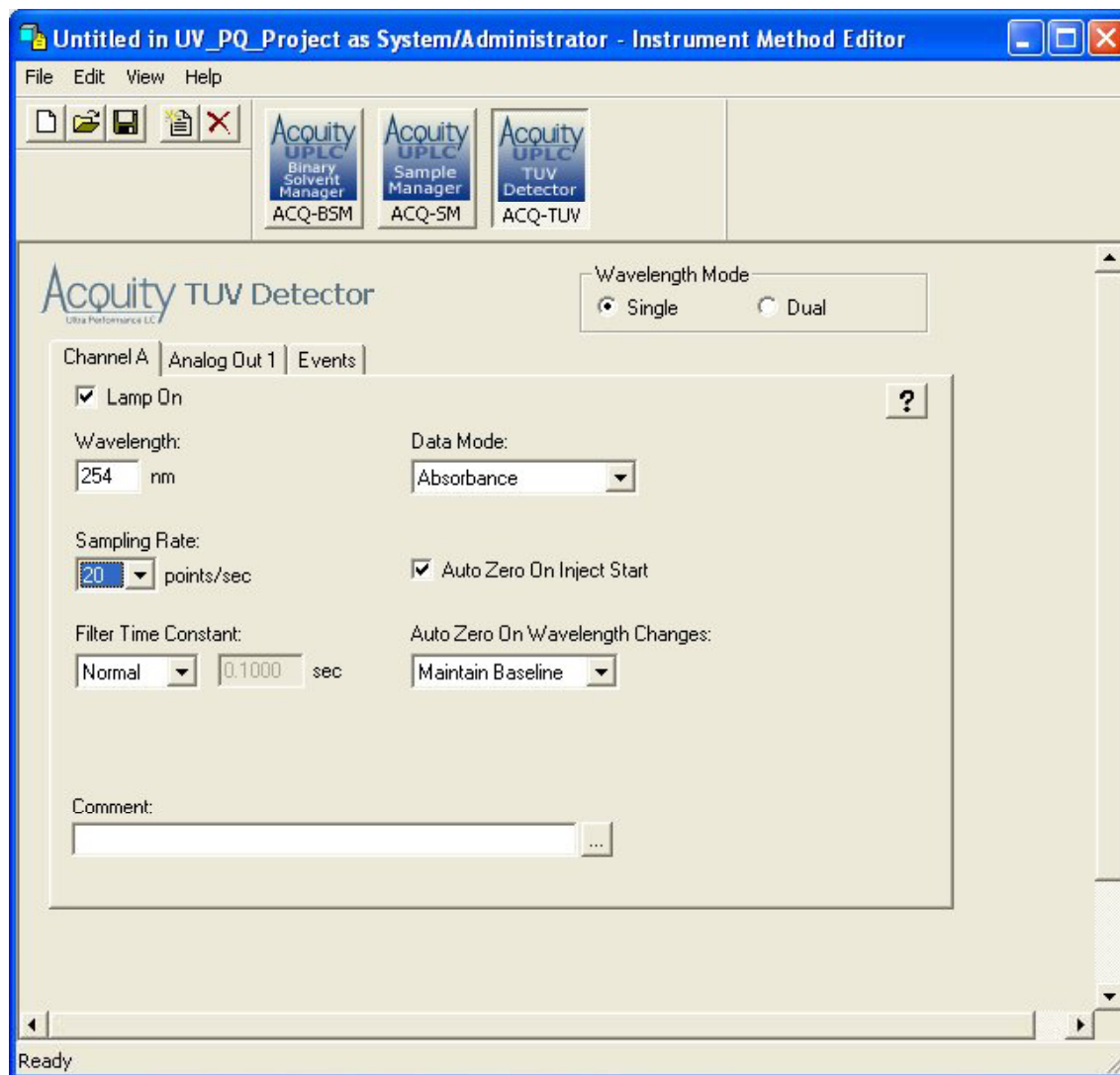
Column 2.1x100mm
400 μ L/min



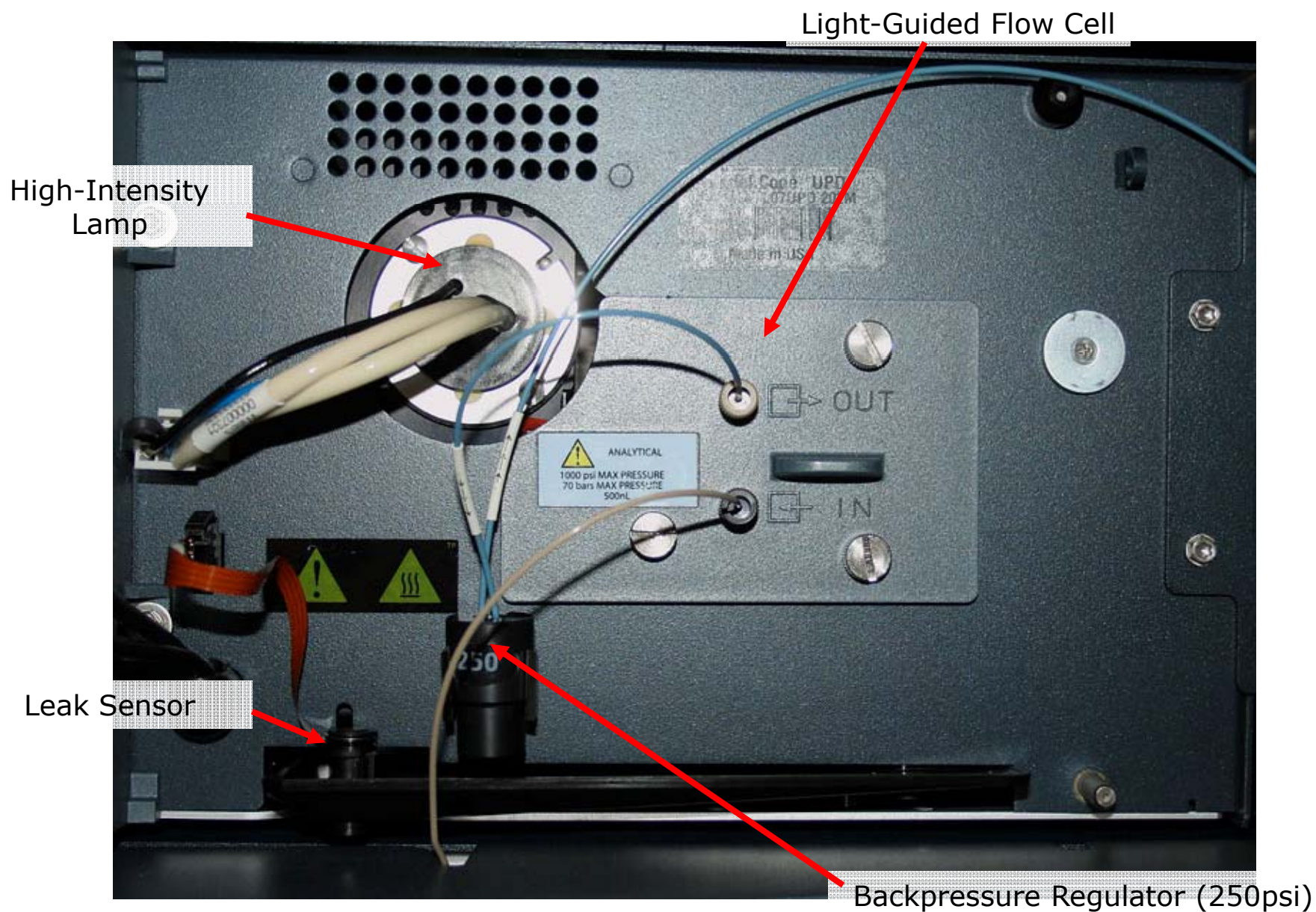
ACQUITY TUV Detector



ACQUITY TUV Method Editor



ACQUITY PDA Detector



ACQUITY PDA Method Editor

Modify ACQUITY PDA Instrument Method

Acquity PDA Detector
Ultra Performance LC

Run Time: 1.00 min

General | 2D Channels | Analog Out | Events

☒ Lamp On

☒ Enable 3D Data

λ Range: 210 nm to 400 nm

Resolution: 1.2 nm

Sampling Rate: 20 points/sec

Filter Time Constant: Normal 0.1000 sec

Exposure Time: Auto msec

☐ Use UV blocking filter (below 210nm)

Comment:

OK

Modify ACQUITY PDA Instrument Method

Acquity PDA Detector
Ultra Performance LC

Run Time: 1.00 min

General | 2D Channels | Analog Out | Events

Data Mode λ

☒ Channel 1 Absorbance 254 1.2 nm resolution

☒ Channel 2 Absorbance 230 1.2 nm resolution

☐ Channel 3

OK Cancel

ACQUITY UPLC System

Flex cart

- Optional UPLC FlexCart
 - Primarily for UPLC use with Mass Spectrometers
- Mobile, adjustable height
- Provides platform for complete UPLC system
 - Electrical outlets
 - Supports PC, monitor, etc.
 - Waste management



End of Presentation