

ACQUITY UPLC® Service Training

Introduction and System Overview

Outline for this Section

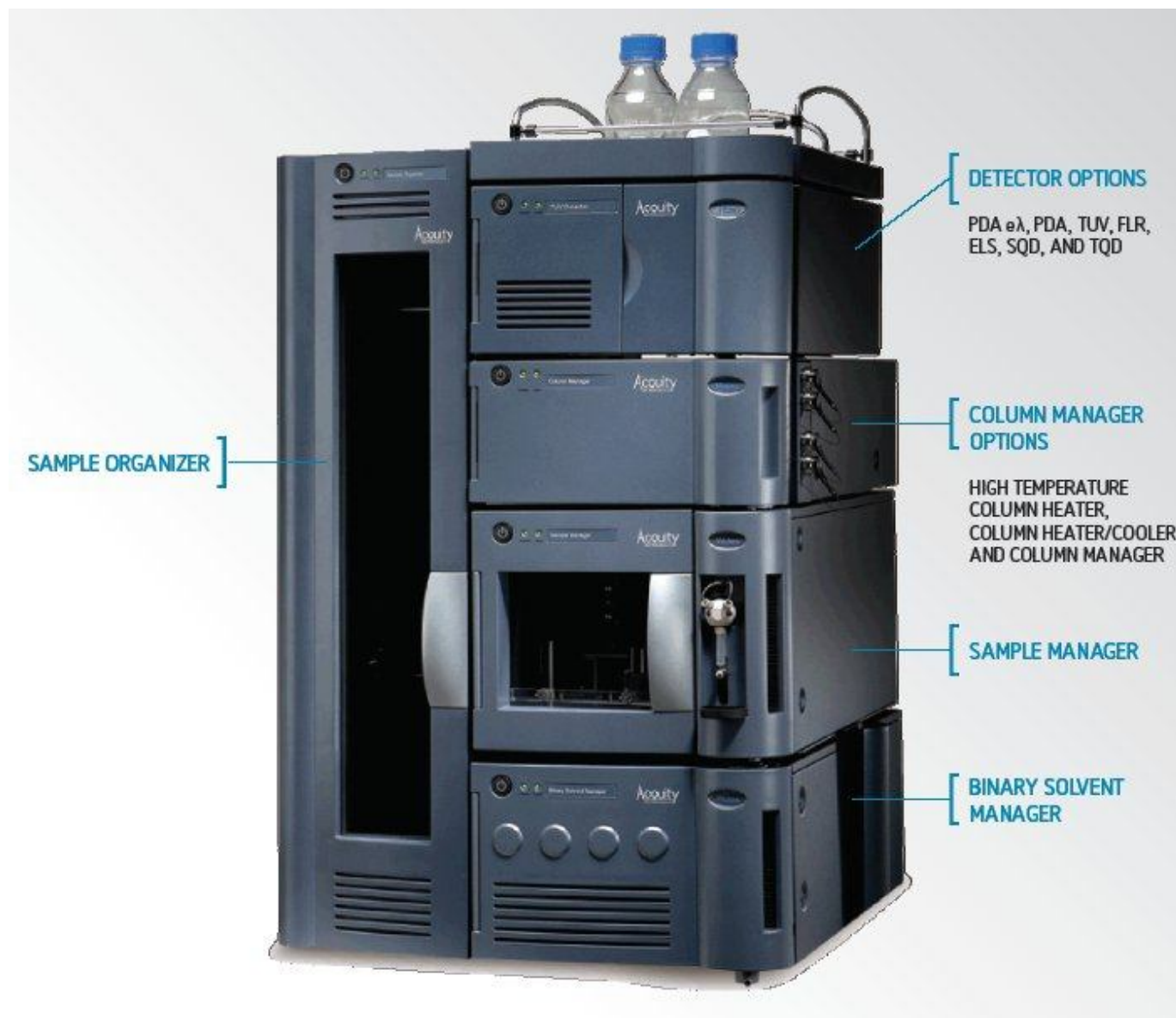
- ACQUITY System Components Introduction
- Brief ACQUITY Chemistry Overview
- ACQUITY Console Tour
- Exercise 1- Console Overview



ACQUITY System Overview

ACQUITY UPLC® Service Training Introduction

ACQUITY UPLC System



ACQUITY UPLC System



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

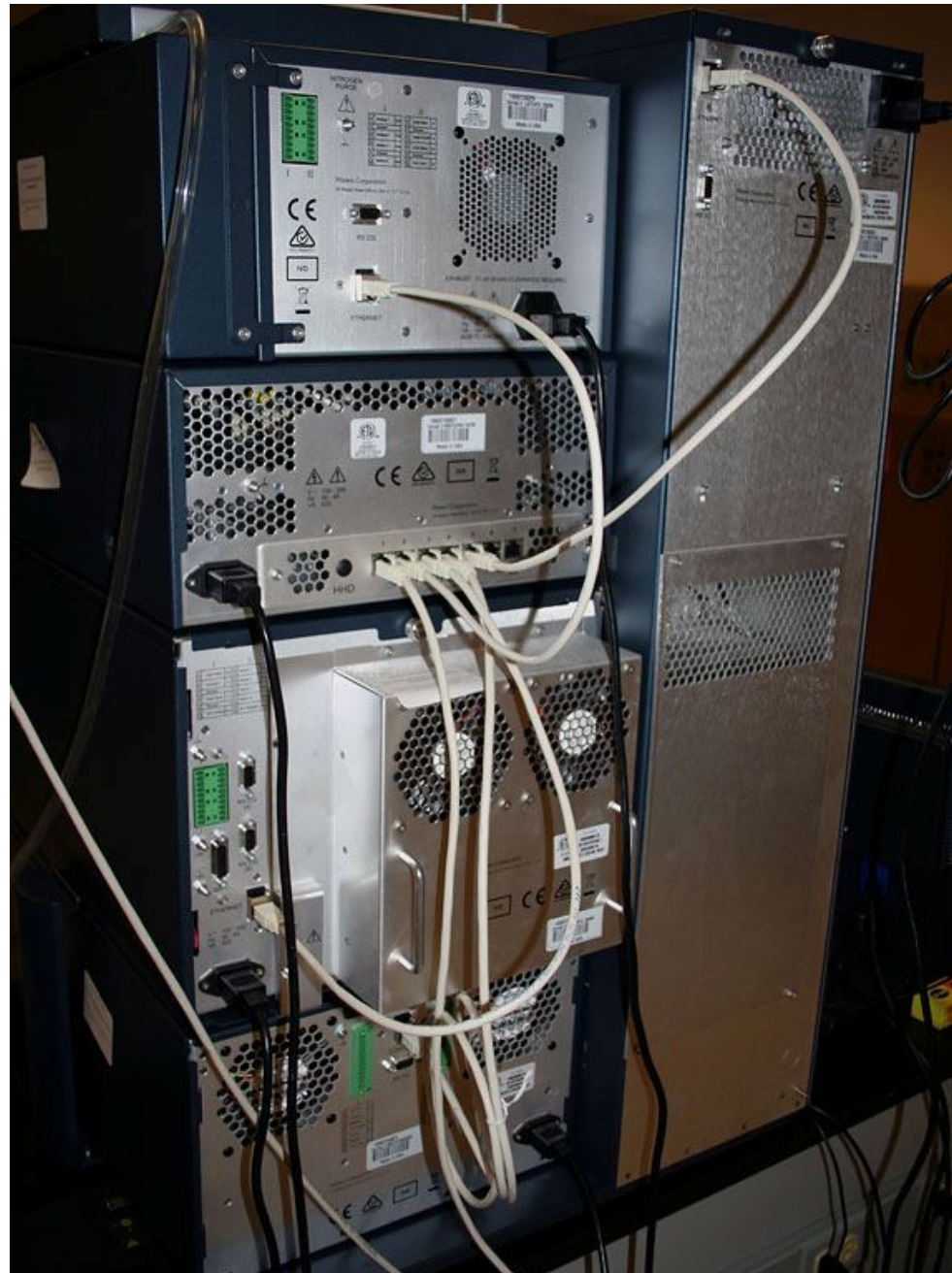
ACQUITY UPLC Detectors

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

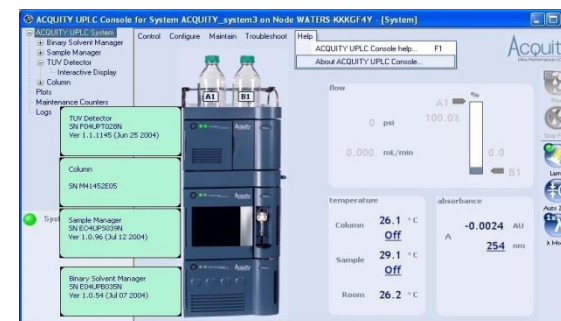
All Modules are Ethernet Controlled



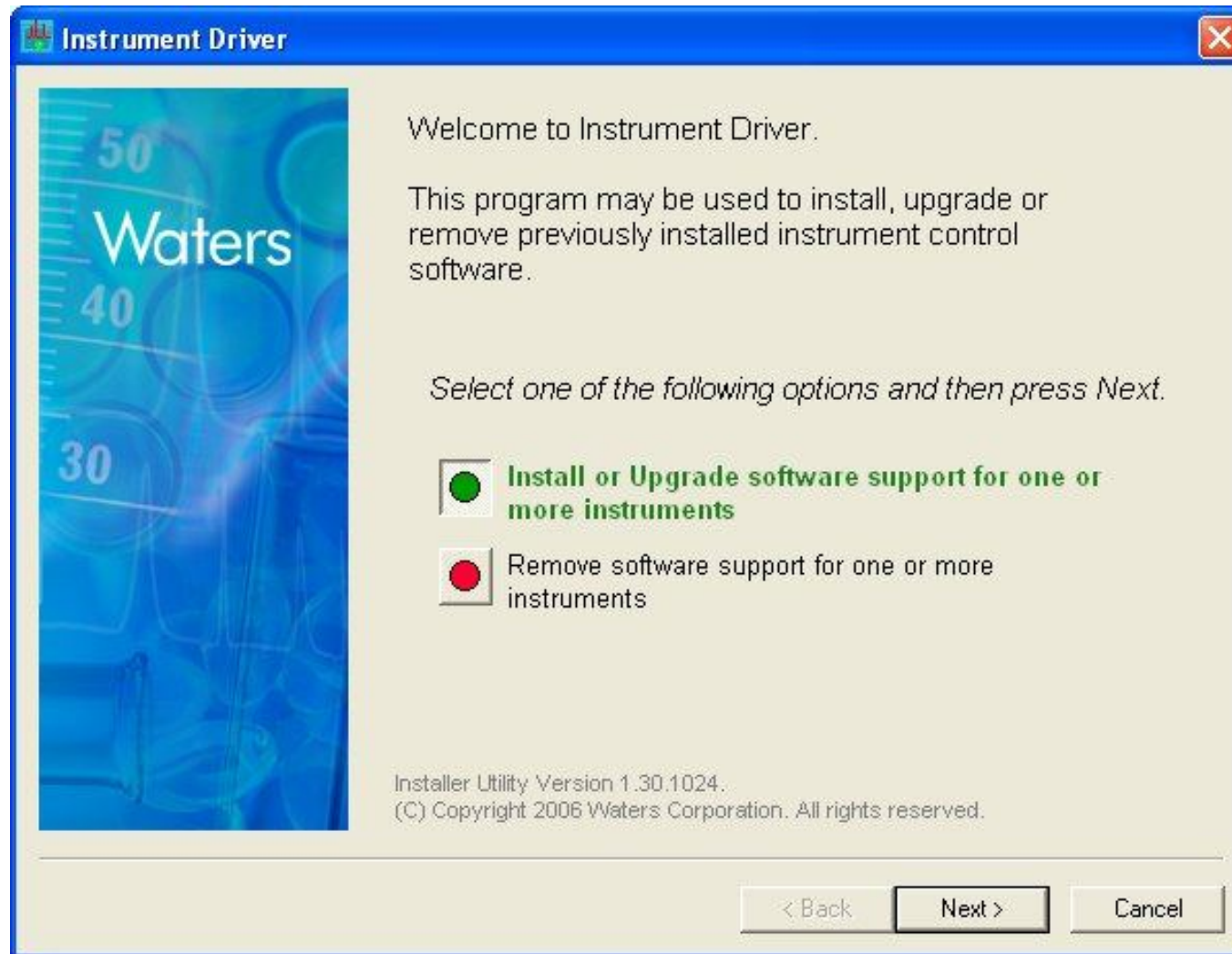
ACQUITY UPLC System

– ACQUITY Console / System Driver

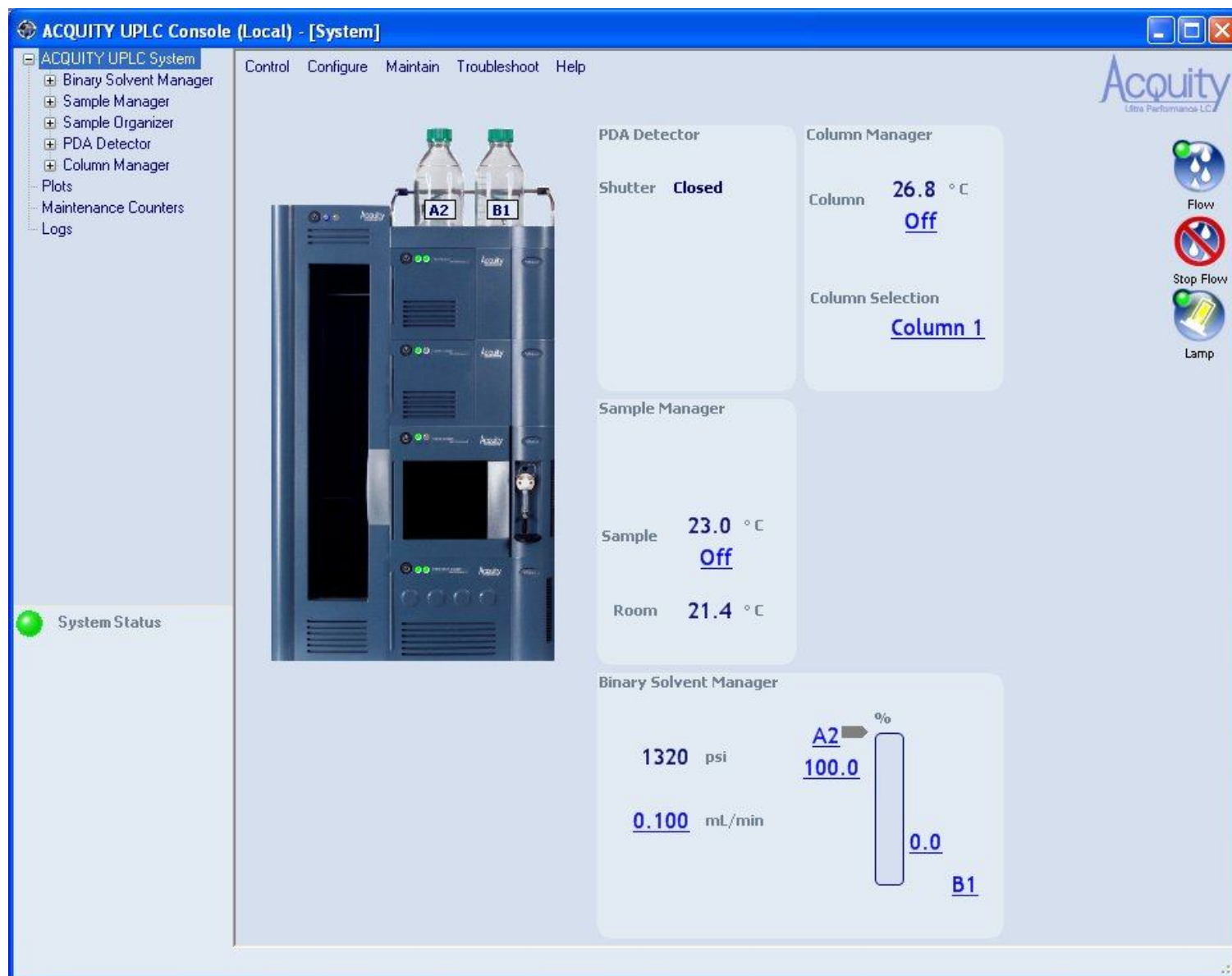
- Plug and Play open architecture
 - Software and firmware packaged together
 - Simple upgrade strategy
- Instrument Control Software
 - Separate portable application folds into:
 - MassLynx
 - Empower 1154 and 2154/Emp3 3471
 - Standalone
 - Other vendor's software
- Minimizes learning curve
 - Simple 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

Ultra Performance LC

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					
-					

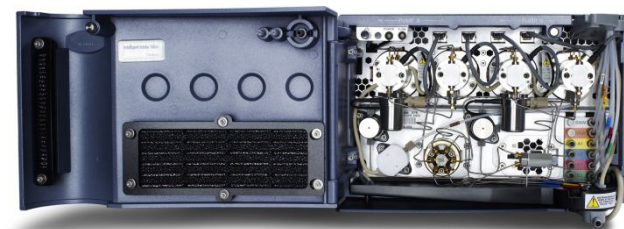
Comment: ...

Ready

ACQUITY UPLC System

Solvent Delivery

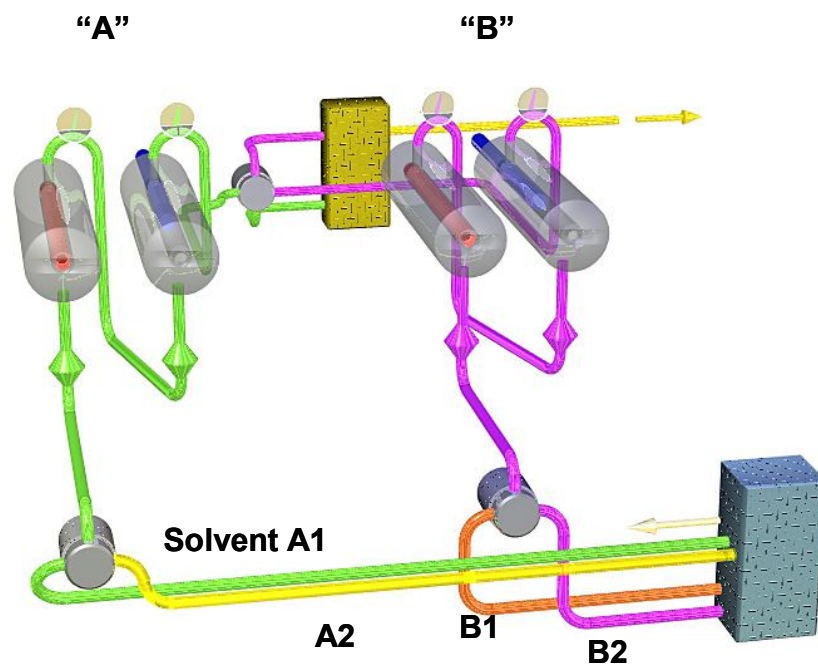
- 15 K psi performance
 - Accurate and precise flow rates
 - Accurate and precise composition
 - Low delay volume for gradient applications
 - <120 μL System Volume
 - Long term reliability



ACQUITY UPLC System

From Serial to Parallel Flow

- Two independently driven **primary** and **accumulator** piston drives
 - Separate drive motors
 - Two transducers
 - Two inlet check valves
- Four Solvents Capability
 - A1 or A2 and B1 or B2
 - Two solvents per gradient
- Six Channel Degasser
 - 4 chromatographic eluents
 - 2 solvents for Sample Manager
 - Strong & weak wash



The image shows the internal components of a laboratory instrument, likely a mass spectrometer or a similar analytical device. The main body is a dark grey or black metal frame. At the top, there are two pump units labeled 'PUMP A' and 'PUMP B'. Each pump has a 'PRI' (primary) and 'ACC' (auxiliary) port. Below the pumps, there are four cylindrical components, possibly solenoid valves or flow meters, arranged in a row. These are connected to a complex network of white and black tubing. In the center, there is a large, clear, cylindrical component, possibly a detector or a sample chamber, with several electrical connections. To the right, there is a control panel with a 'POWER' button (indicated by a green light) and a 'FLOW' button. Various cables and wires are connected to the system, including a blue cable labeled 'B2' and a yellow cable labeled 'B1'. A warning label is visible on the right side, indicating a high voltage or electrical hazard.

ACQUITY UPLC Tubing & Fittings

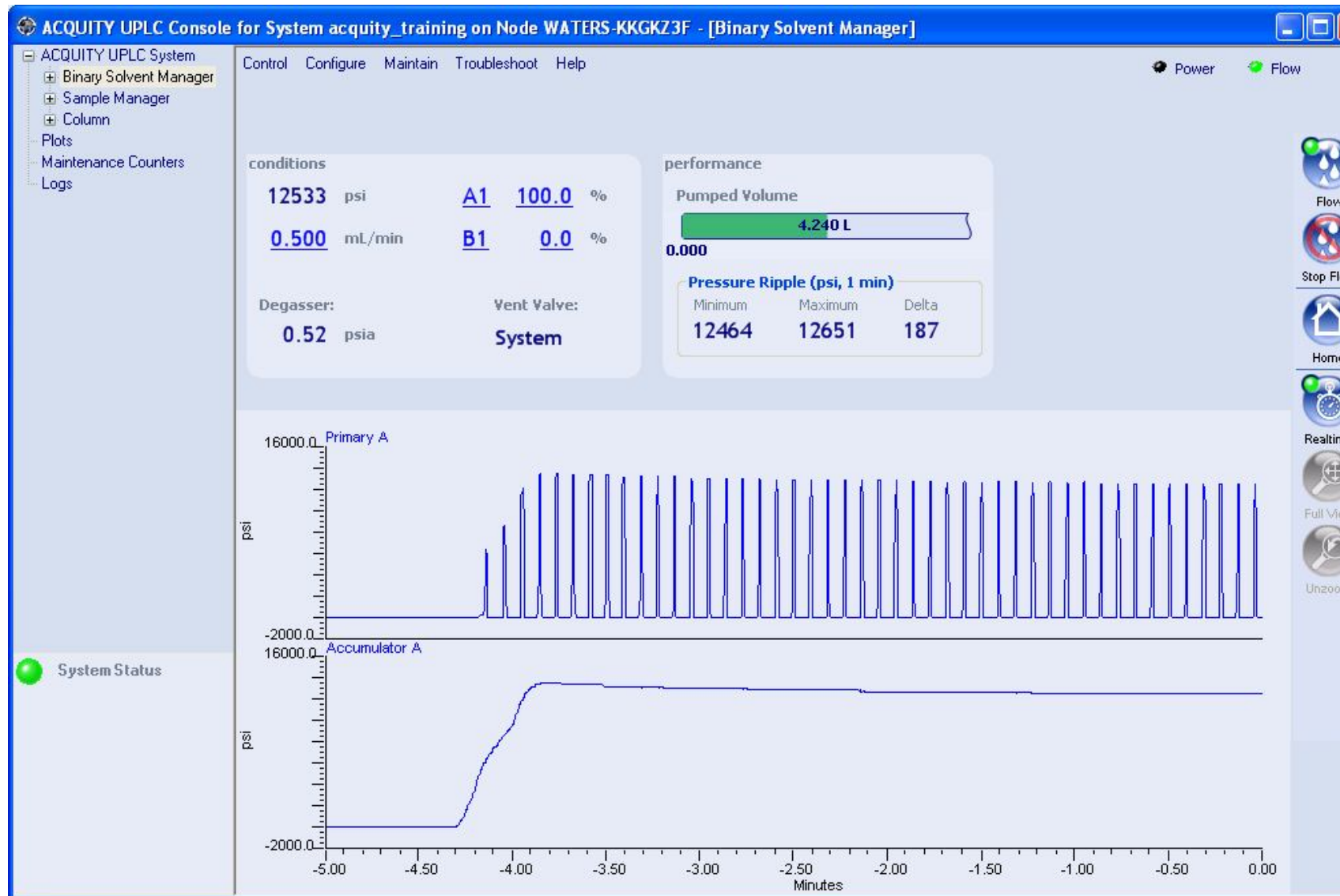
- Center-less ground tubing



- Gold Compression Screw
- 2 Piece Swagelock Ferrules



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

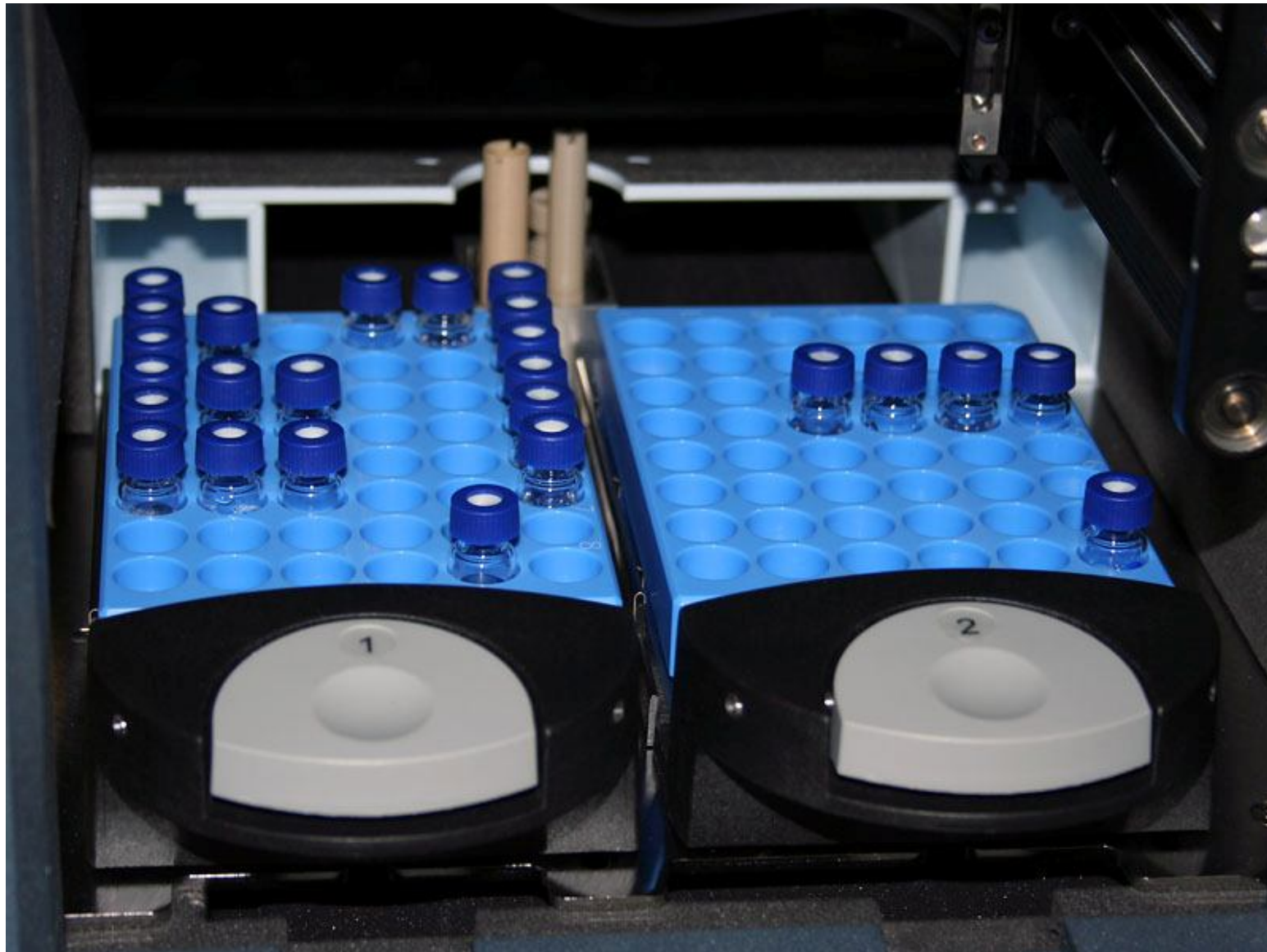
ACQUITY UPLC System

Sample Management

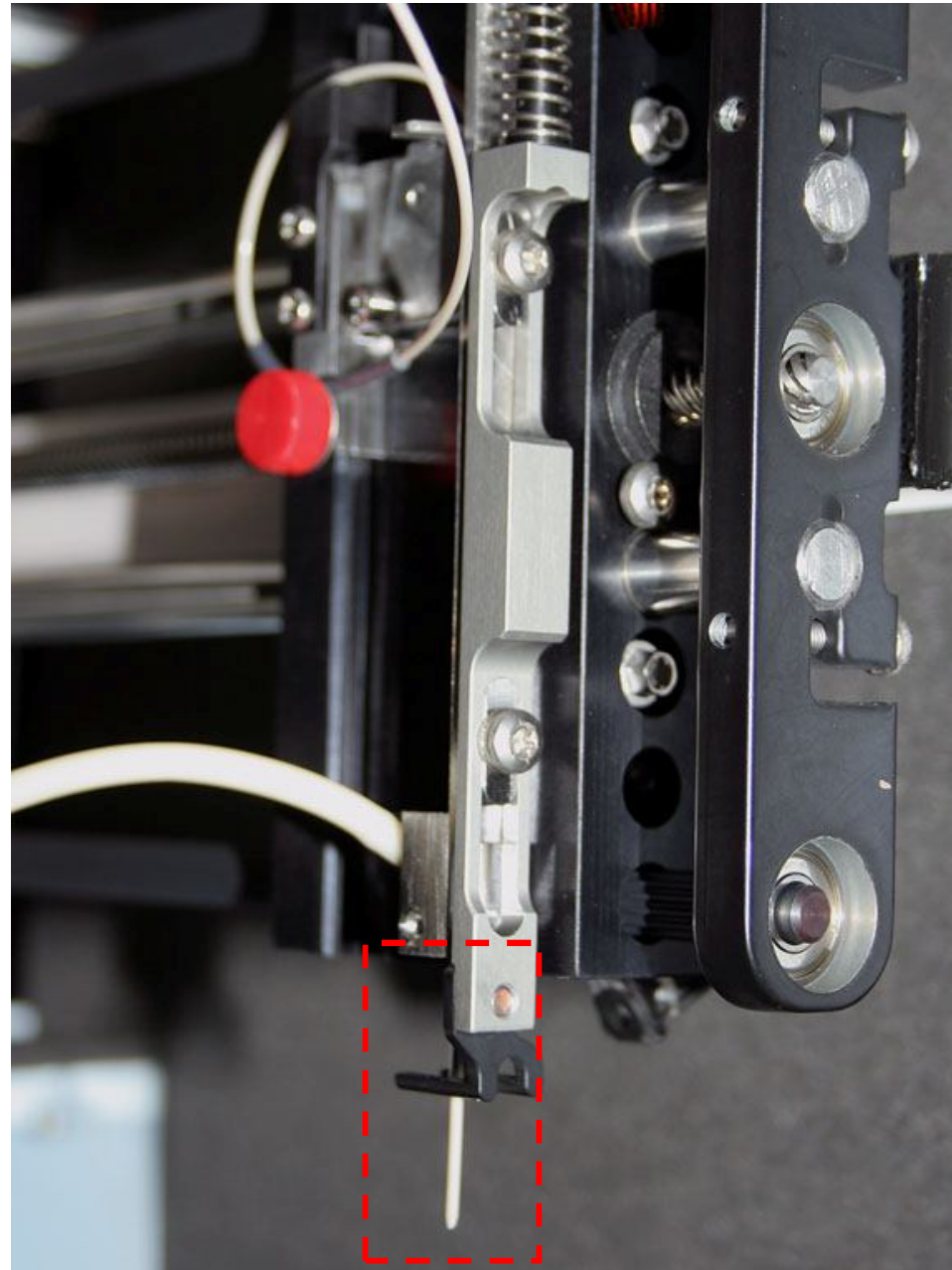
- Fast cycle times
 - High speed injection sequence
- Smaller scale
 - Carryover <0.005%
 - Dual wash
 - 1 through 50 μL loops
- High pressure
 - Rugged valve switching design
 - Withstand high pressure actuation
 - No impact on column life
- XYZZ Sample transport



Supports Two ANSI STD Plates in XY Plane



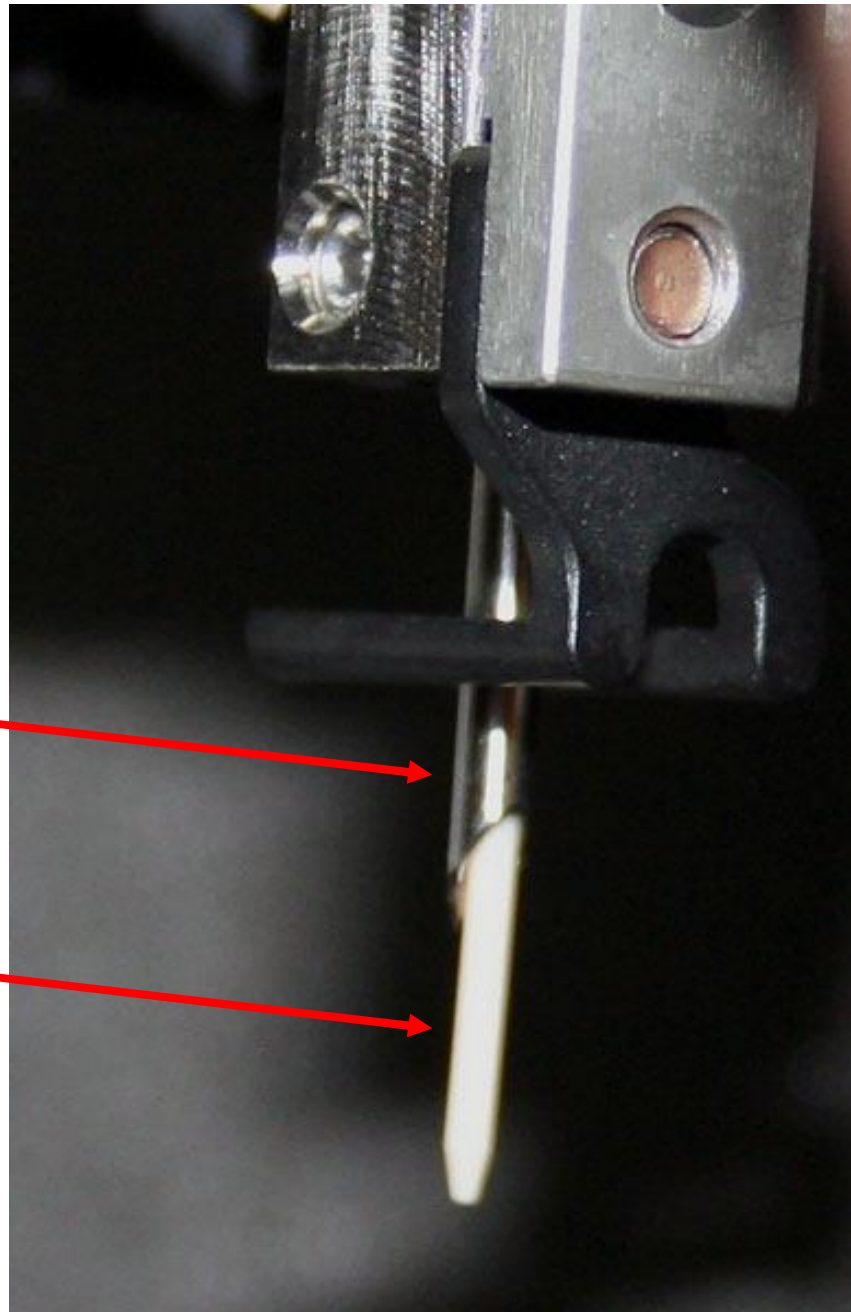
Separate Piercing and Sampling Needles (Zz Axis)



Separate Piercing and Sampling Needles

Piercing Needle

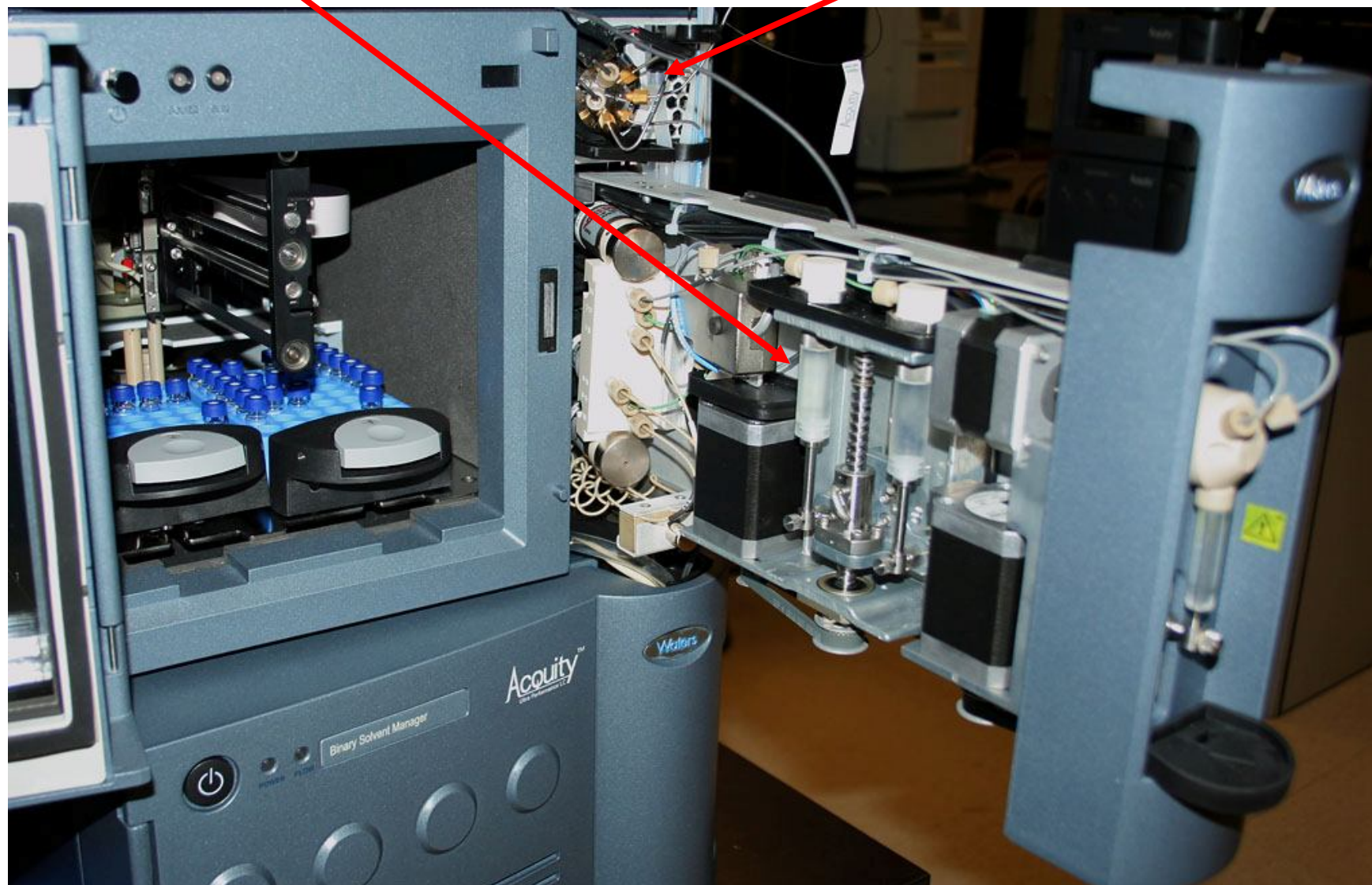
Sample Needle



Sample Manager Fluidics- Loop Based Injector

Fluidics Draw

Everflow Inject Valve



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: Disable min

☐ Load Ahead

Wash Solvents

Weak: 10/90 ACN/Water ... 600 μL

Strong: 100% Acetonitrile ... 200 μL

Temperature

Column: Off $^{\circ}\text{C}$ Alarm Band: ☐ $\pm$ 5.0 $^{\circ}\text{C}$

Sample: 25.0 $^{\circ}\text{C}$ ☒ $\pm$ 5 $^{\circ}\text{C}$

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



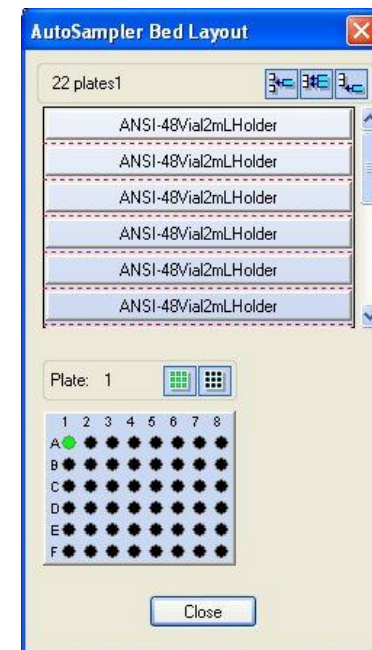
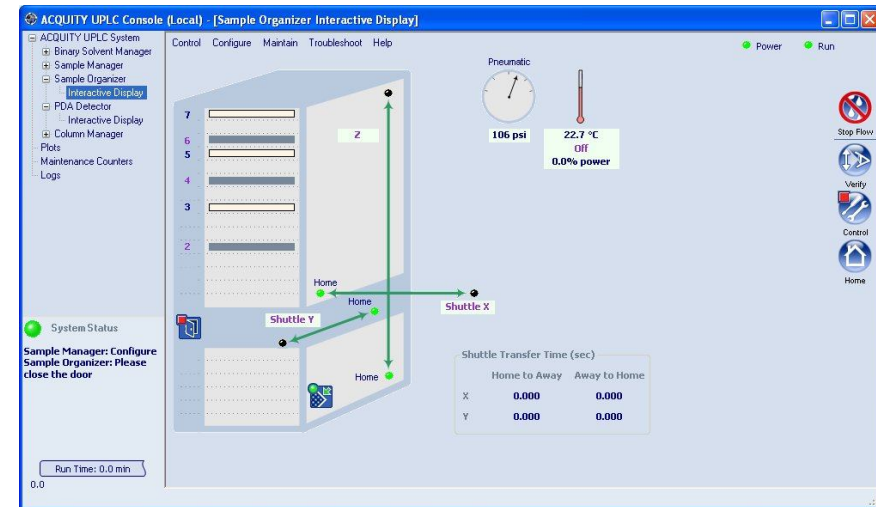
ACQUITY UPLC System

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



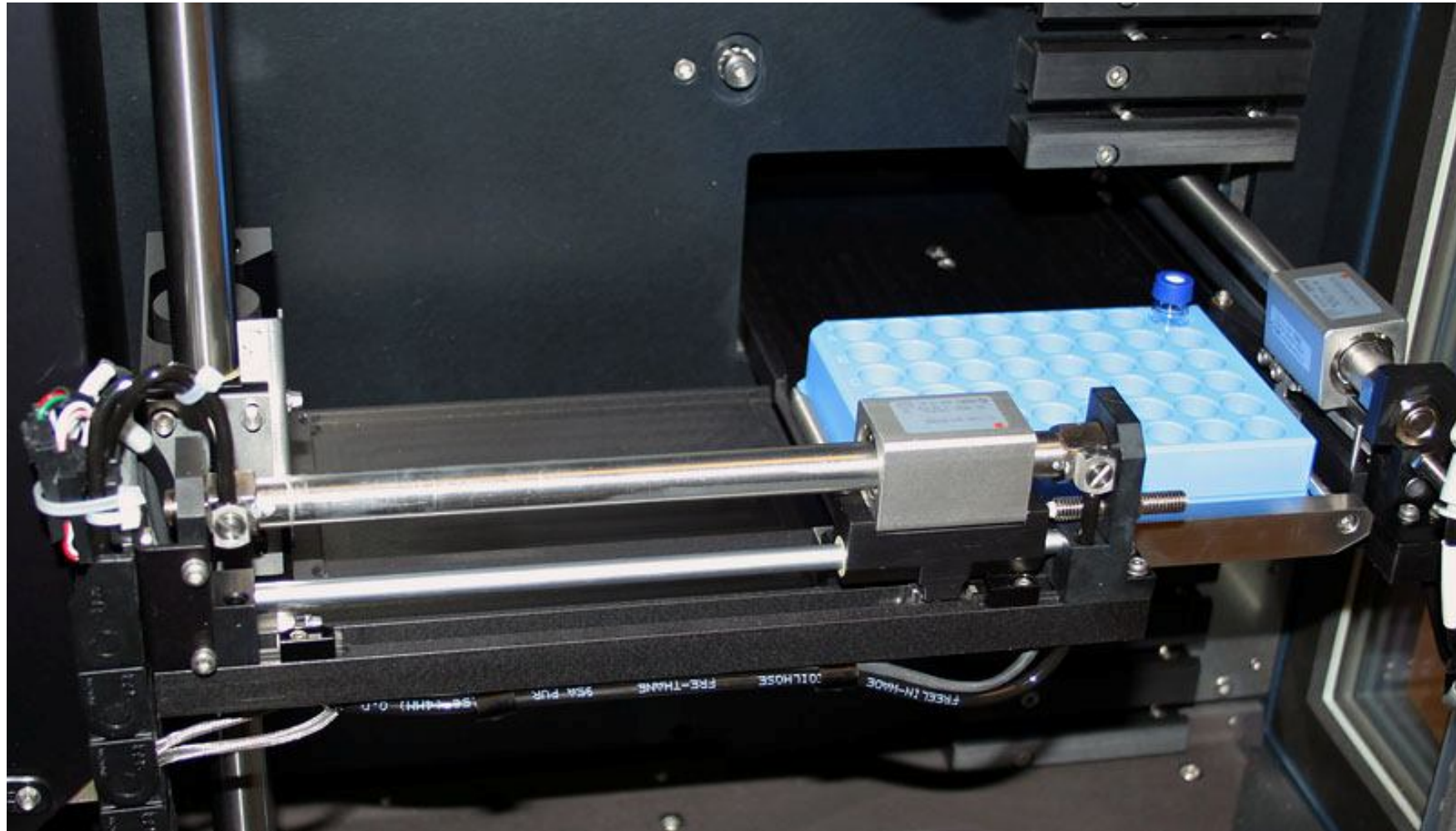
Sample Organizer



Sample Organizer- Open Side-View



Pneumatic-Driven Transport Shuttles



SM Configuration with Sample Organizer



ACQUITY UPLC System

3 Column Management Configurations

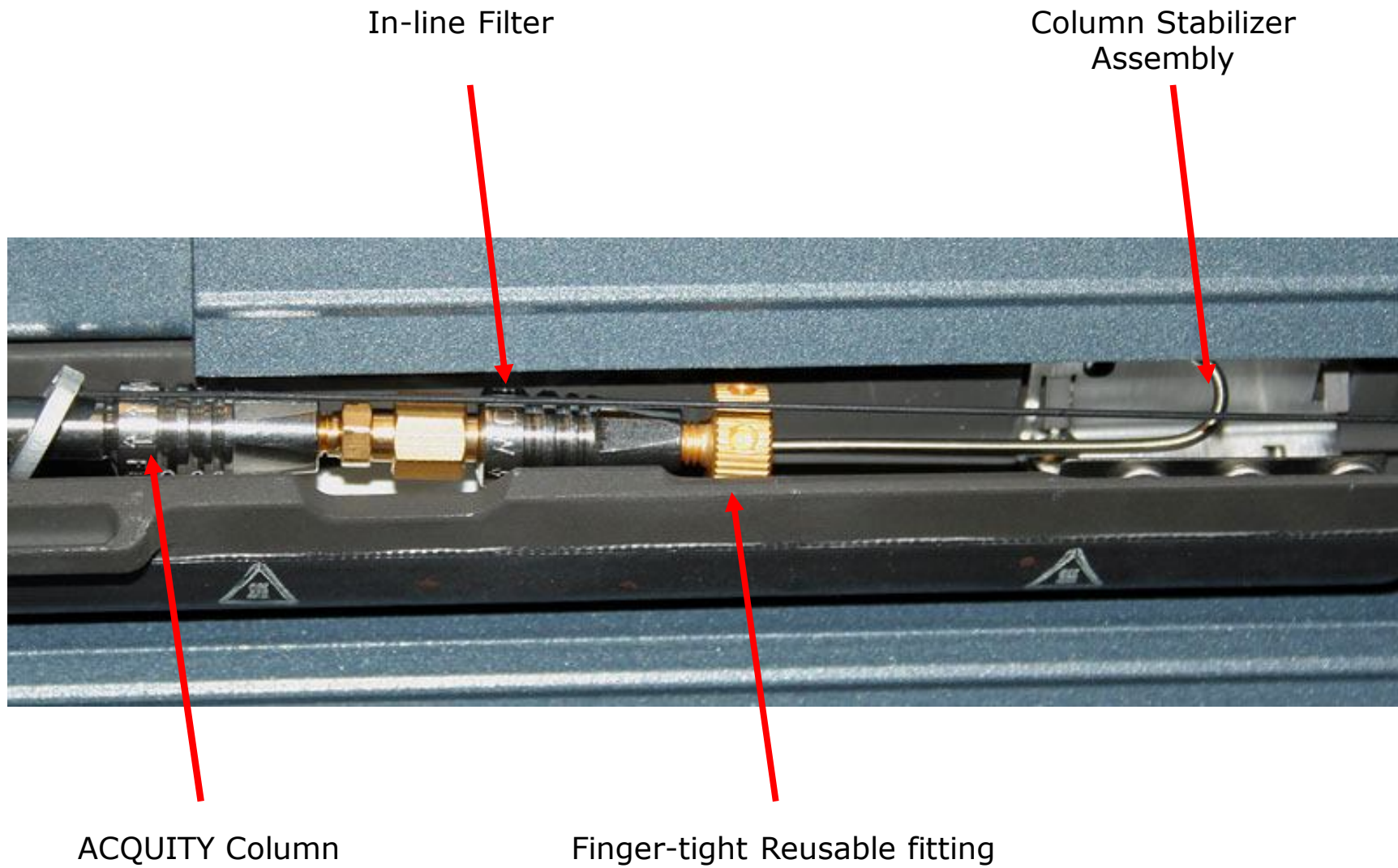
1. Sample Manager w/ Column Heater Module
 - Heating only- Ambient to 90°C
 2. Sample Manager w/ Column Heater/Cooler Module
 - Heating and cooling- 10°C to 90°C
 3. Sample Manager w/ Column Manager
 - Heating and cooling 10°C to 90°C + 2 valves
 - 4 column selection
 - Bypass column and divert to waste
- ACQUITY UPLC 30 cm Column Heater/Cooler
 - Accessory with the three system option above



HT Column Heater



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
Disable

☐ Load Ahead

Wash Solvents

Weak: 10/90 ACN/Water 600 µL

Strong: 100% Acetonitrile 200 µL

Temperature

Column: Off °C Alarm Band: ☐ ± 5.0 °C

Sample: 25.0 °C ☒ ± 5 °C

Comment:

Advanced...

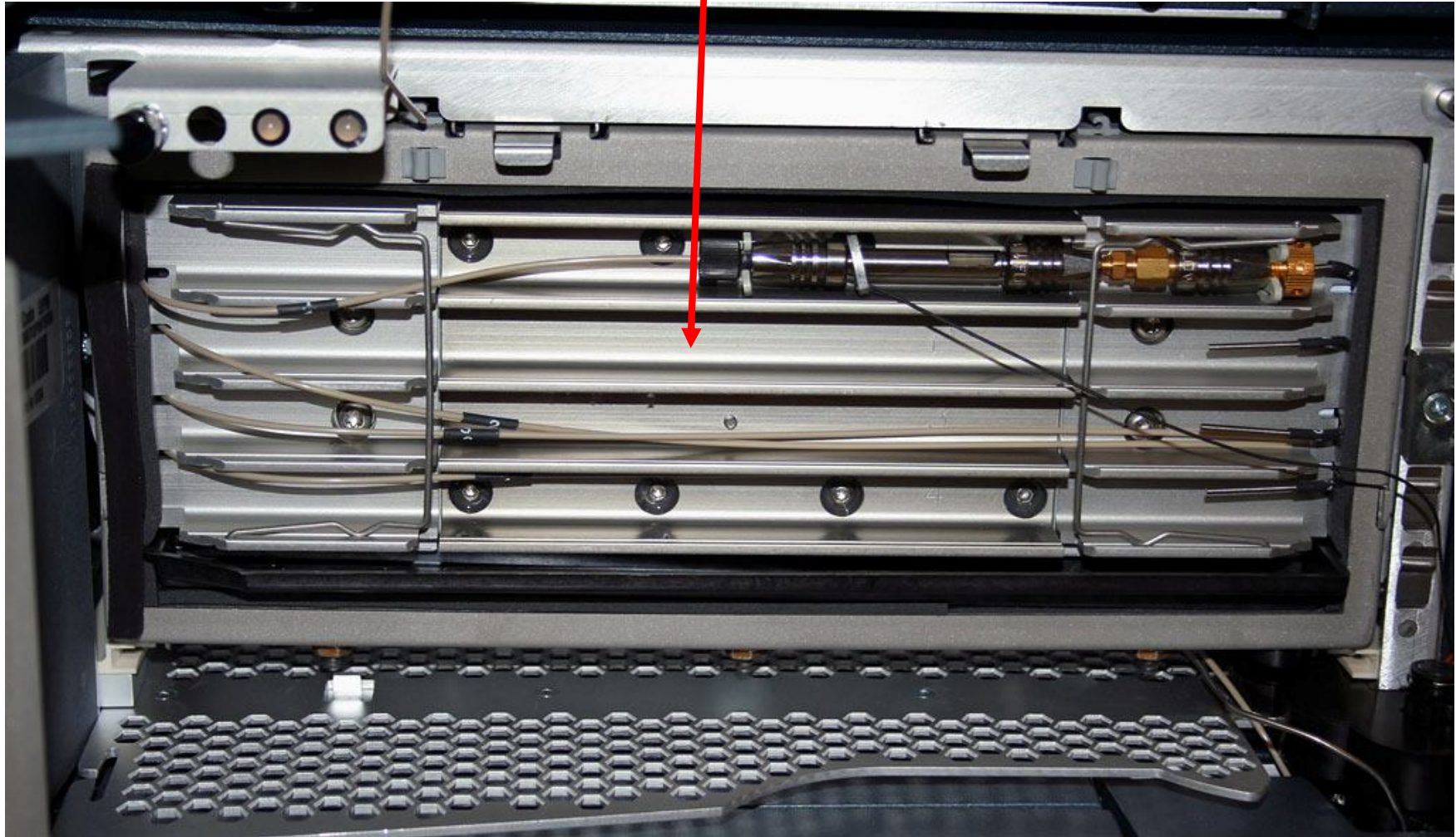
OK Cancel

Column Heater eCord Reader



Column Manager

Four Separate Column Compartments



Two Switching Valves (internally mounted)

Column Manager eCord Readers



Column 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**

Heater

Temperature: 25.0 °C

☒ Alarm Band: ± 5.0 °C

Column Selection

Valve Position: Column 1

Equilibration Time: 0.1 min

☐ Acquire column temperature data channel

OK Cancel

ACQUITY UPLC 30cm Column Heater/Cooler

To accommodate two 30cm HPLC Columns



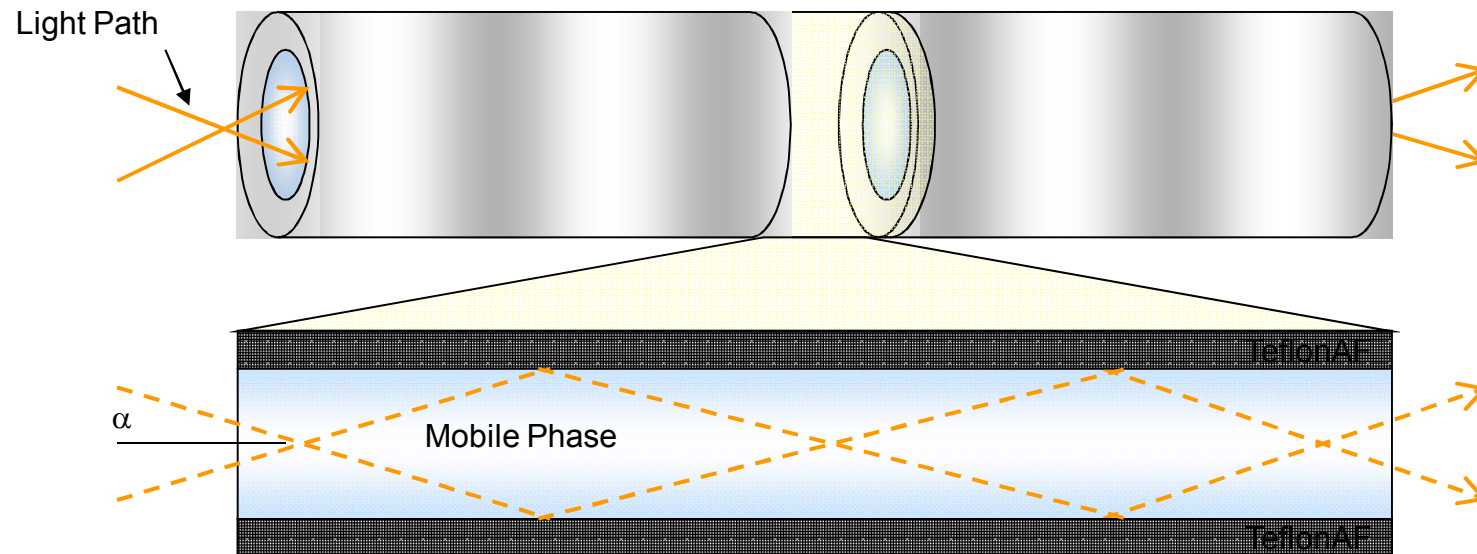
ACQUITY UPLC System

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
 - Coming soon:
 - Acquity RI Detector (2012)
 - Acquity ECD (TBD=To Be Determined)



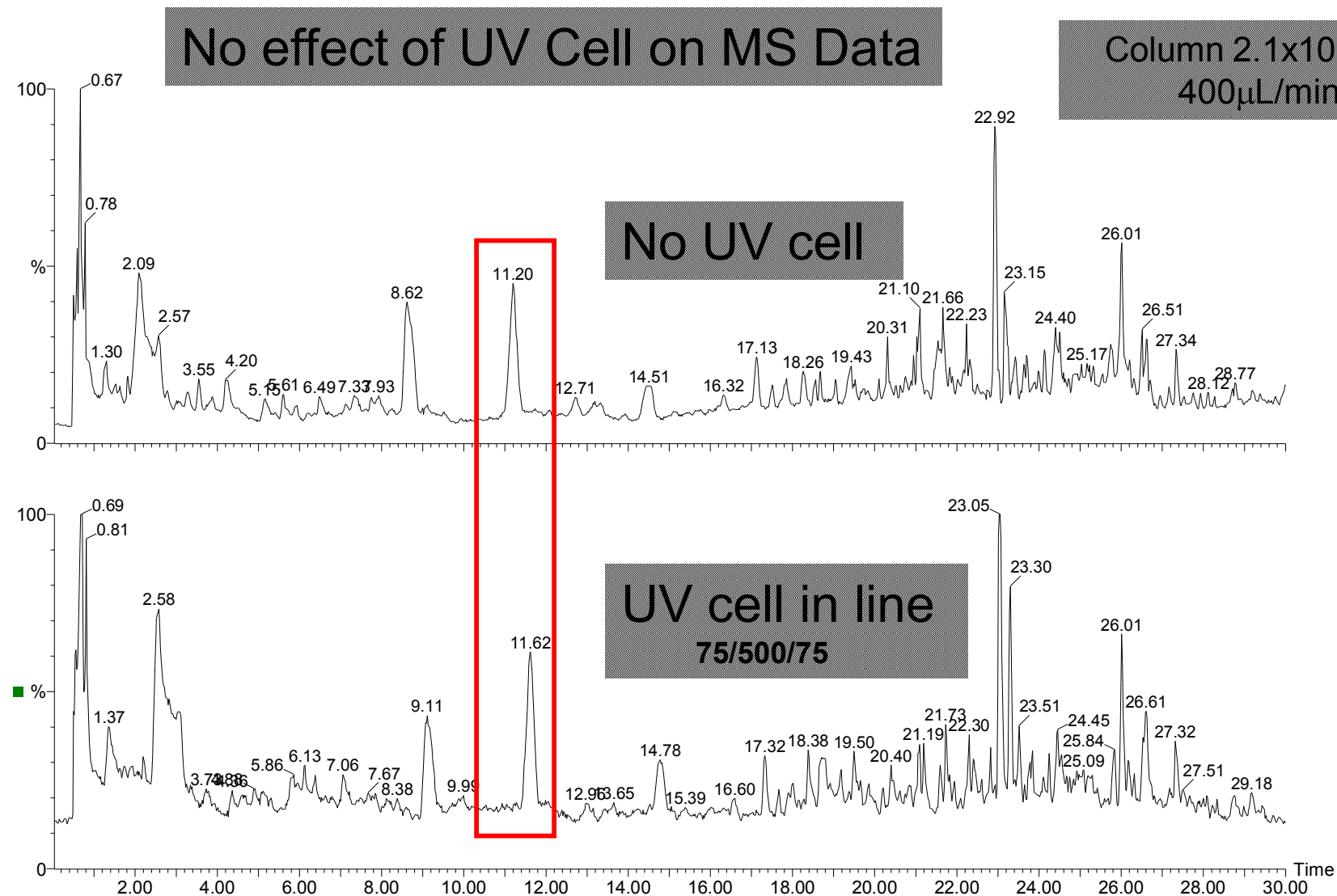
Light Guided Flow Cells- Total Internal Reflection



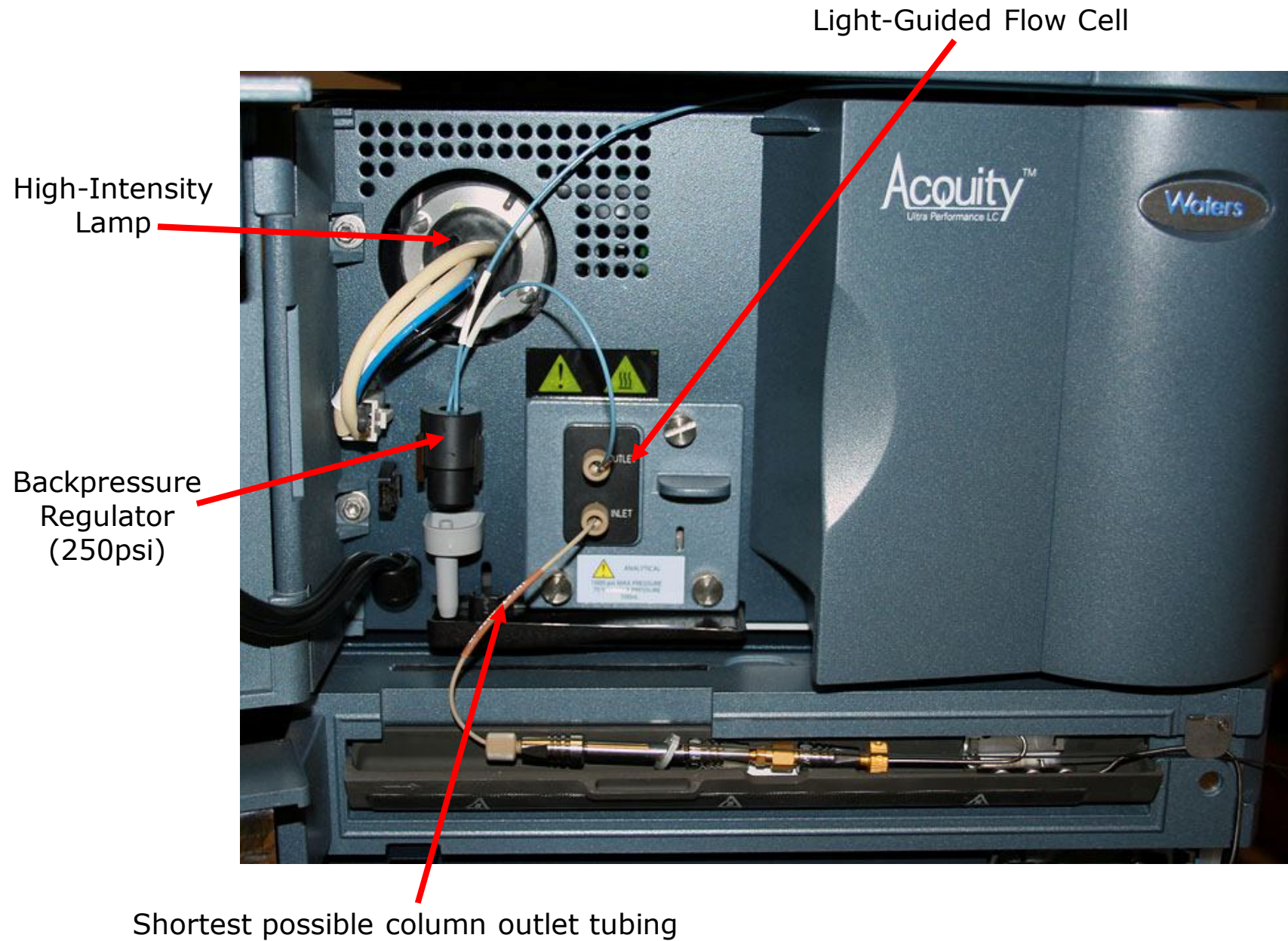
- Light rays entering the liquid core of the flow cell are internally reflected when they meet the Teflon AF boundary.
 - These rays are transmitted through the cell without loss, except for absorption by the sample
- Allows for good sensitivity while keeping flow cell volume (and consequently dispersion)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

ACQUITY UPLC System

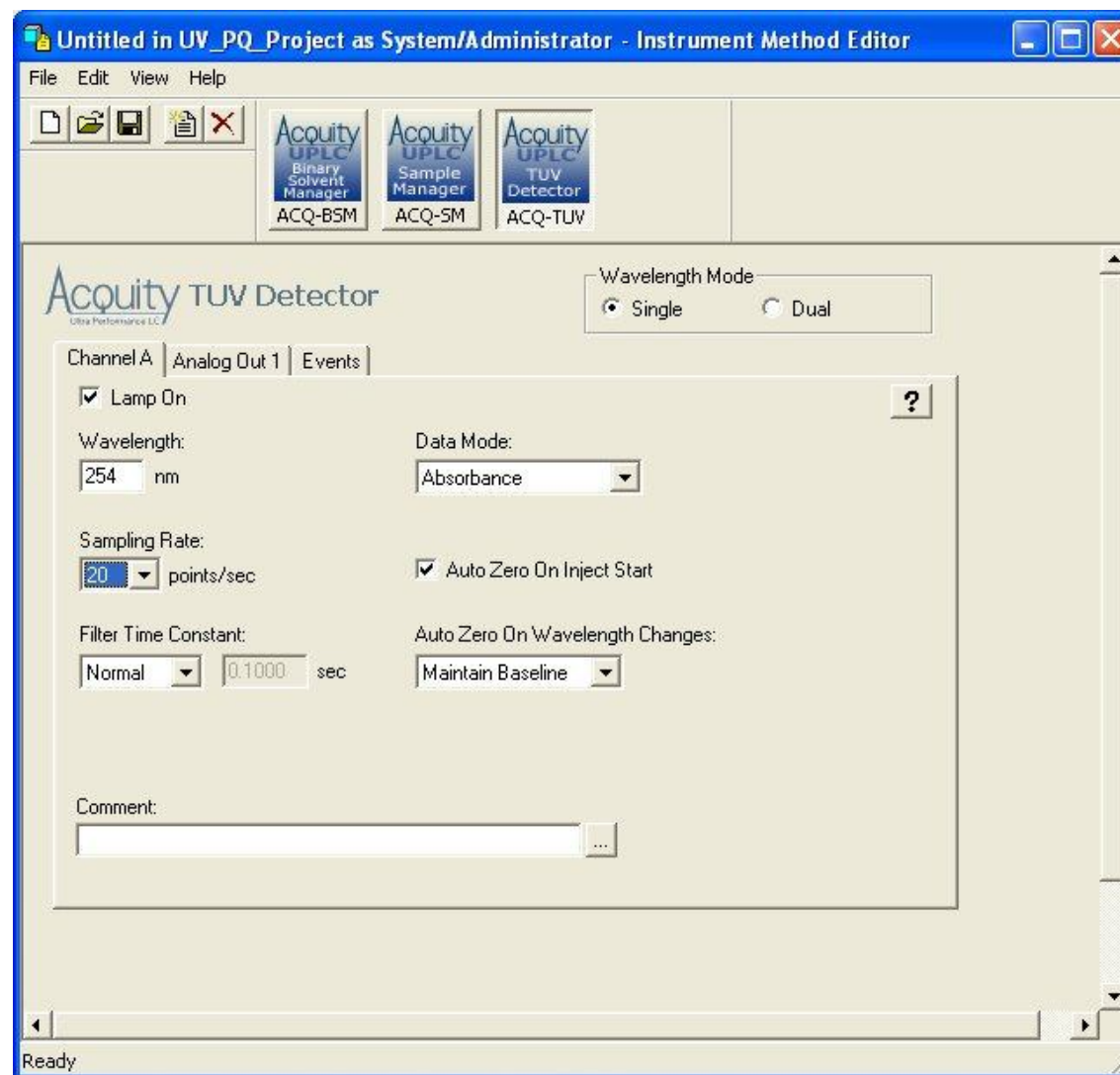
Low dispersion UPLC flow cells



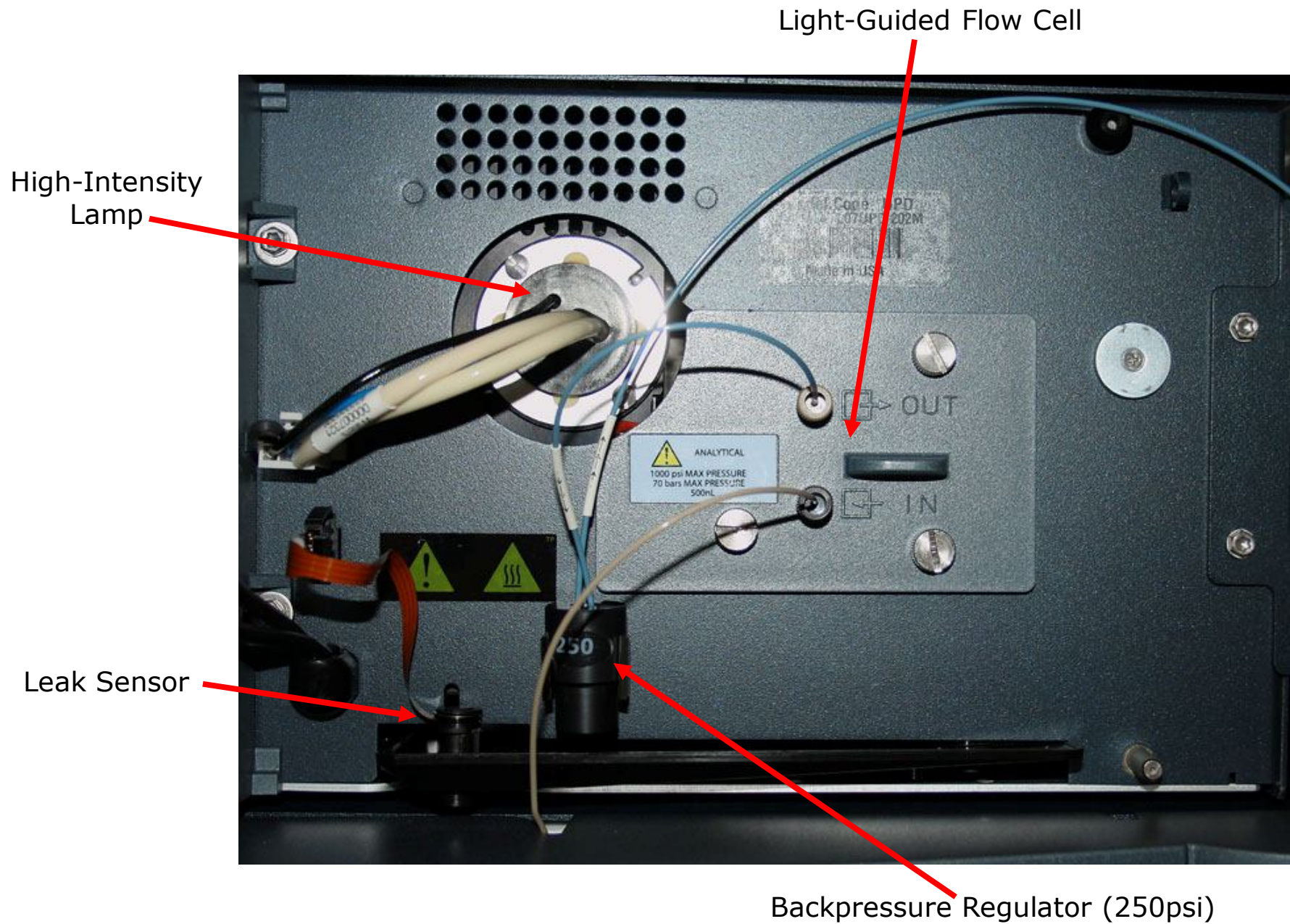
ACQUITY TUV Detector



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

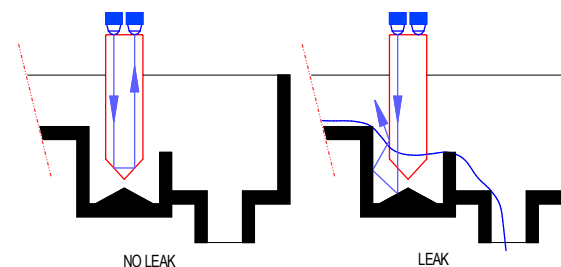
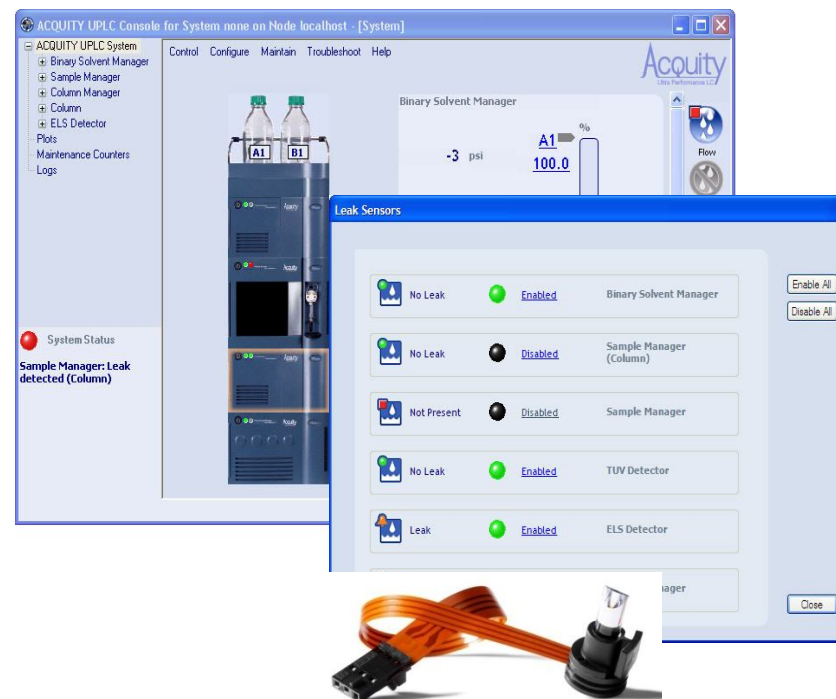
☒ Channel 2 Absorbance 230 1.2 nm resolution

☐ Channel 3

OK Cancel

ACQUITY UPLC System- *Leak Sensors*

- The design is simple
- Sensors are mounted in new black drip trays in the ACQUITY UPLC System modules
- The Leak Sensors can be reset quickly and easily
- They work with any solvent supported with ACQUITY UPLC Systems
 - They were tested with high viscosity solvents such as Hexane



Leak Detector Setup for the ACQUITY PDA



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



Chemistry Overview

ACQUITY UPLC® Service Training Introduction

ACQUITY Column Engineering

The image shows a detailed view of an ACQUITY UPLC column assembly, including the column body, frits, and a coiled tube. Callout lines point from various parts of the assembly to descriptive text boxes.

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 eCord technology

COLUMN PACKING

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

ACQUITY UPLC columns feature eCord technology

The bottom of the image displays two screenshots of the ACQUITY UPLC software interface. The left screenshot shows the 'ACQUITY UPLC Column' configuration window with fields for 'Column Name', 'Part Number', 'Batch Number', 'Packing Method', 'Packing Date', 'Packing Location', 'Packing Operator', 'Packing Instrument', 'Packing Pressure', 'Packing Temperature', 'Packing Humidity', 'Packing Time', 'Packing Location', 'Packing Operator', 'Packing Instrument', 'Packing Pressure', 'Packing Temperature', and 'Packing Humidity'. The right screenshot shows the 'Chromatographic Results' window with a chromatogram plot and a table of peak data.

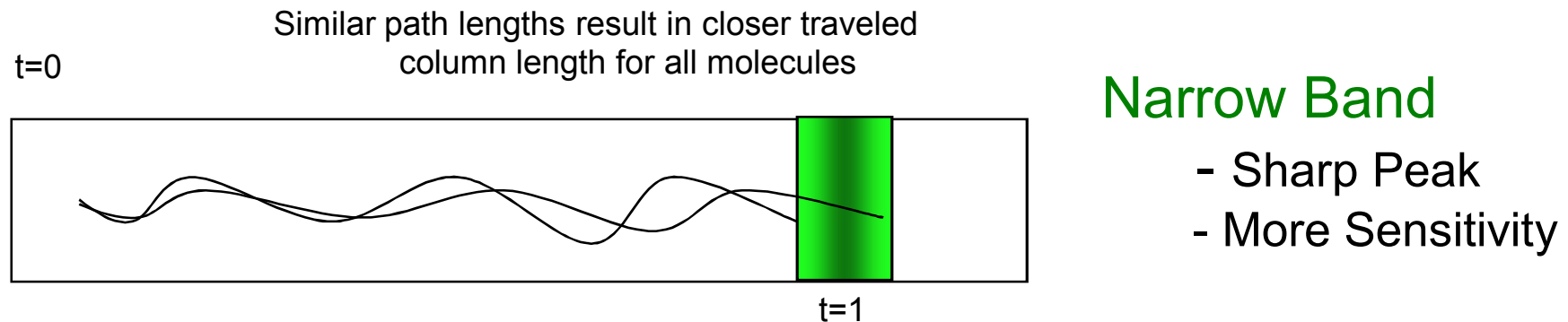
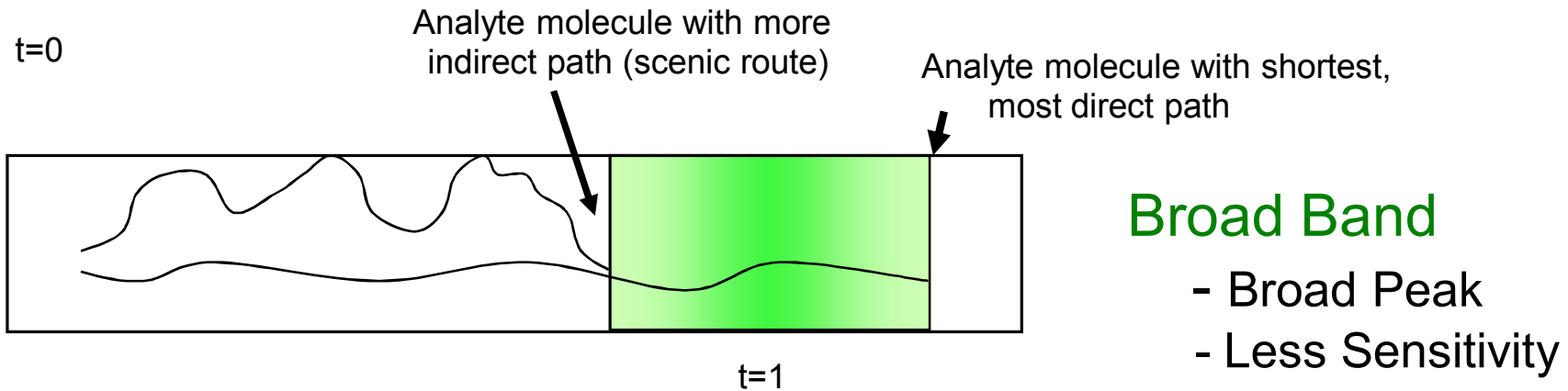
Peak	Retention Time (min)	Area	Height	Width	Asymmetry	Tailing Factor
1	1.112	1.112	1.112	1.112	1.112	1.112
2	1.112	1.112	1.112	1.112	1.112	1.112
3	1.112	1.112	1.112	1.112	1.112	1.112
4	1.112	1.112	1.112	1.112	1.112	1.112
5	1.112	1.112	1.112	1.112	1.112	1.112

*From the ACQUITY UPLC Columns Brochure

UPLC™

- Analytical separations are driven by and limited by the column chemistry
- Particle size is a primary consideration in column performance
 - Smaller particle size means higher pressure
 - Smaller particle size means higher efficiency, greater capacity
- Holistic design for the ACQUITY UPLC™ System
 - Enables the small particle packing to be fully utilized (15,000 psi)
 - Enables the detection of smaller amounts of material – redesigned cells, faster data analysis rates
 - Ensures reduced carryover sample to sample
 - Enables multiple samples can be run

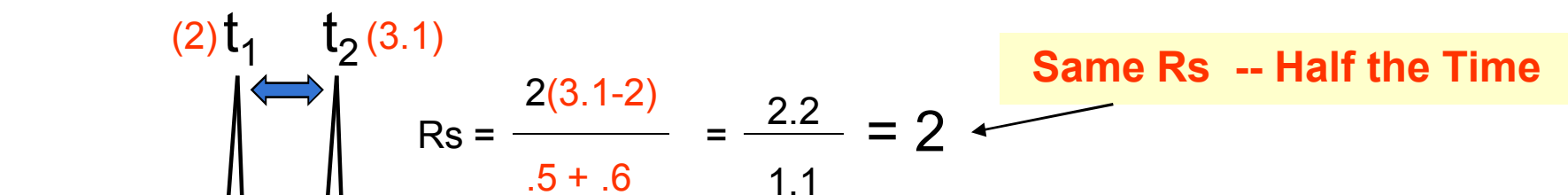
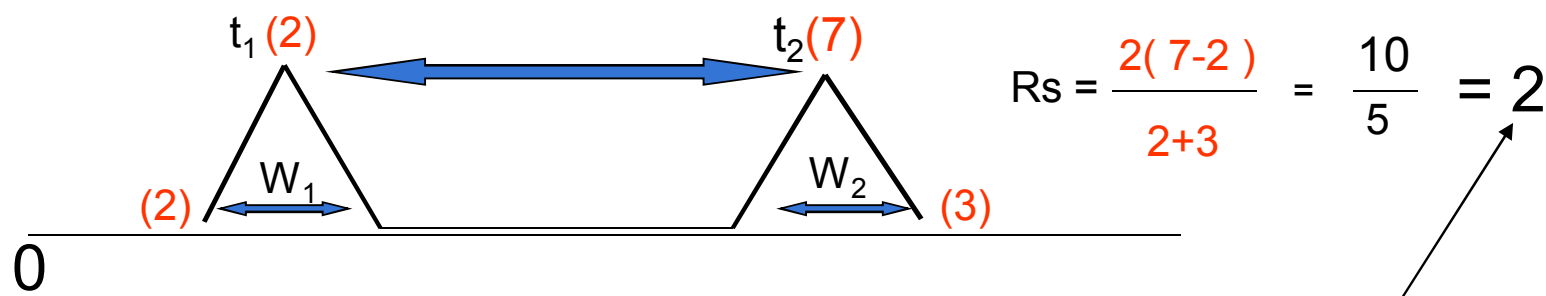
Band Broadening of an Analyte Band - Chromatographic Conditions make the Difference



van Deemter terms explain what happens

Achieving Resolution and Speed in Chromatographic Separations

$$R_s = \frac{2(t_2 - t_1)}{W_1 + W_2}$$



Narrow Peaks (smaller W) allows you to maintain Resolution and Go Faster!

W ↓ N ↑

The van Deemter Equation and “Plates”

van Deemter Equation

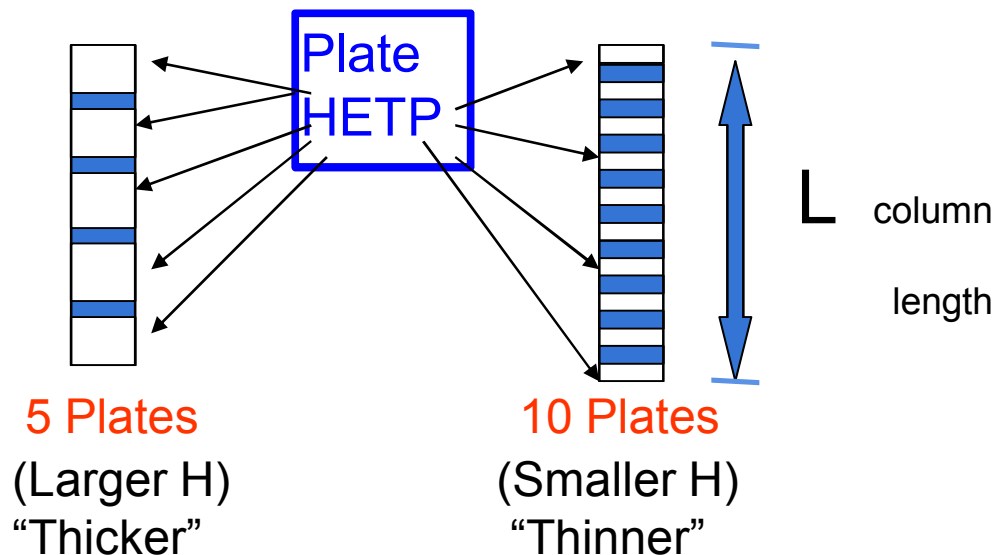
$$H = \text{A Term} + \text{B Term} + \text{C Term}$$

$H = \text{HETP}$ (Height Equivalent to Theoretical Plate)

$$N = \frac{L}{\text{HETP}}$$

$N = \# \text{ Plates}$

(From Distillation Theory)



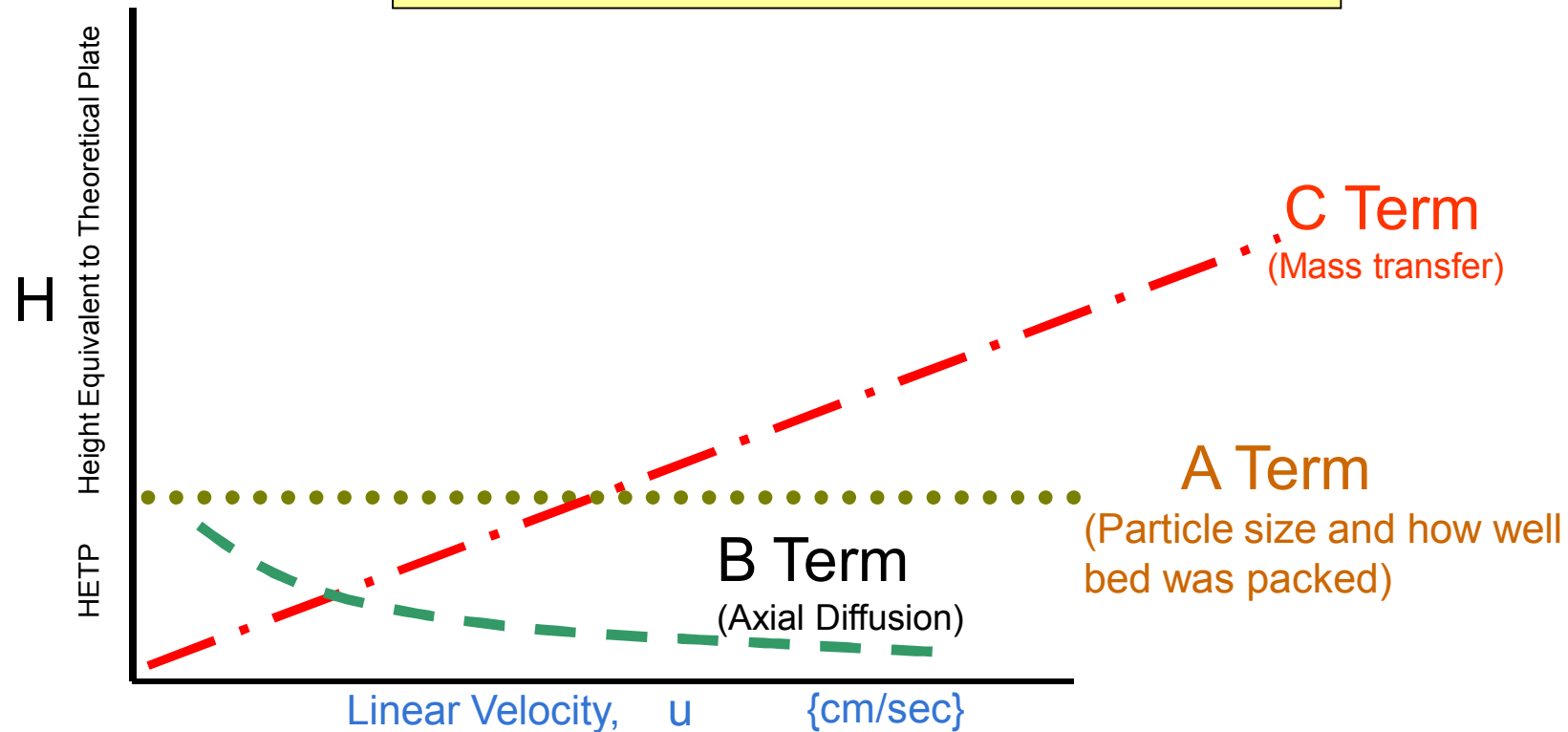
[Need H to be as Small as Possible so you can “fit” more “plates” into a column]

Particle Size and Flow Rate

van Deemter Equation

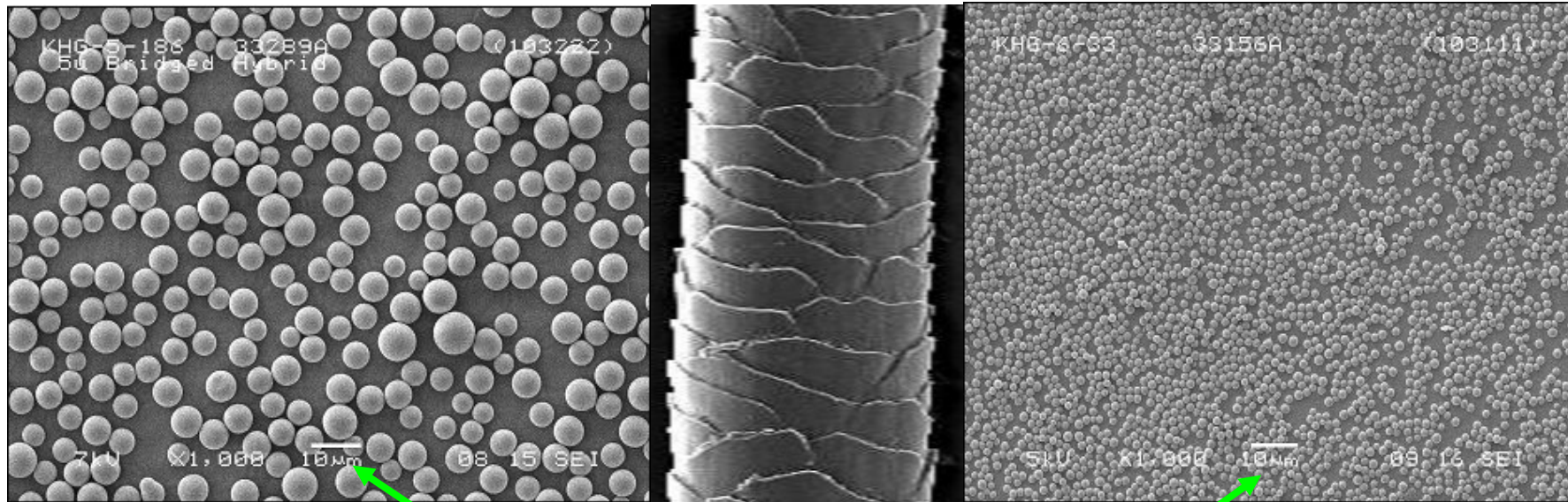
A term + B term + C term

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



A Term- Particle Size and Packing Efficiency

60 μm Human Hair
(very fine hair)



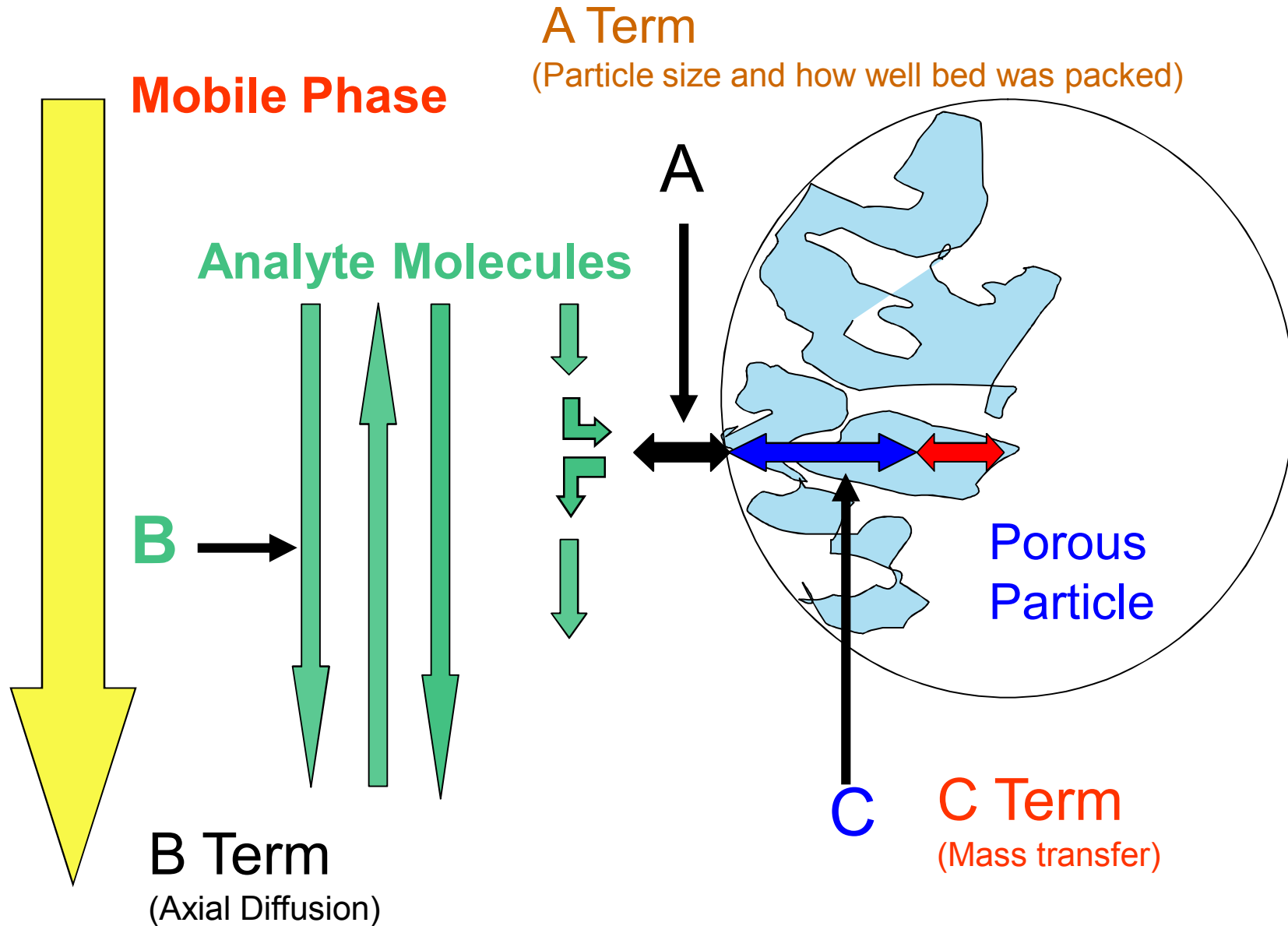
5 μm
Analytical Particles
(can fit 12 across hair)

Images are on
same scale
(Bar = 10 μm)

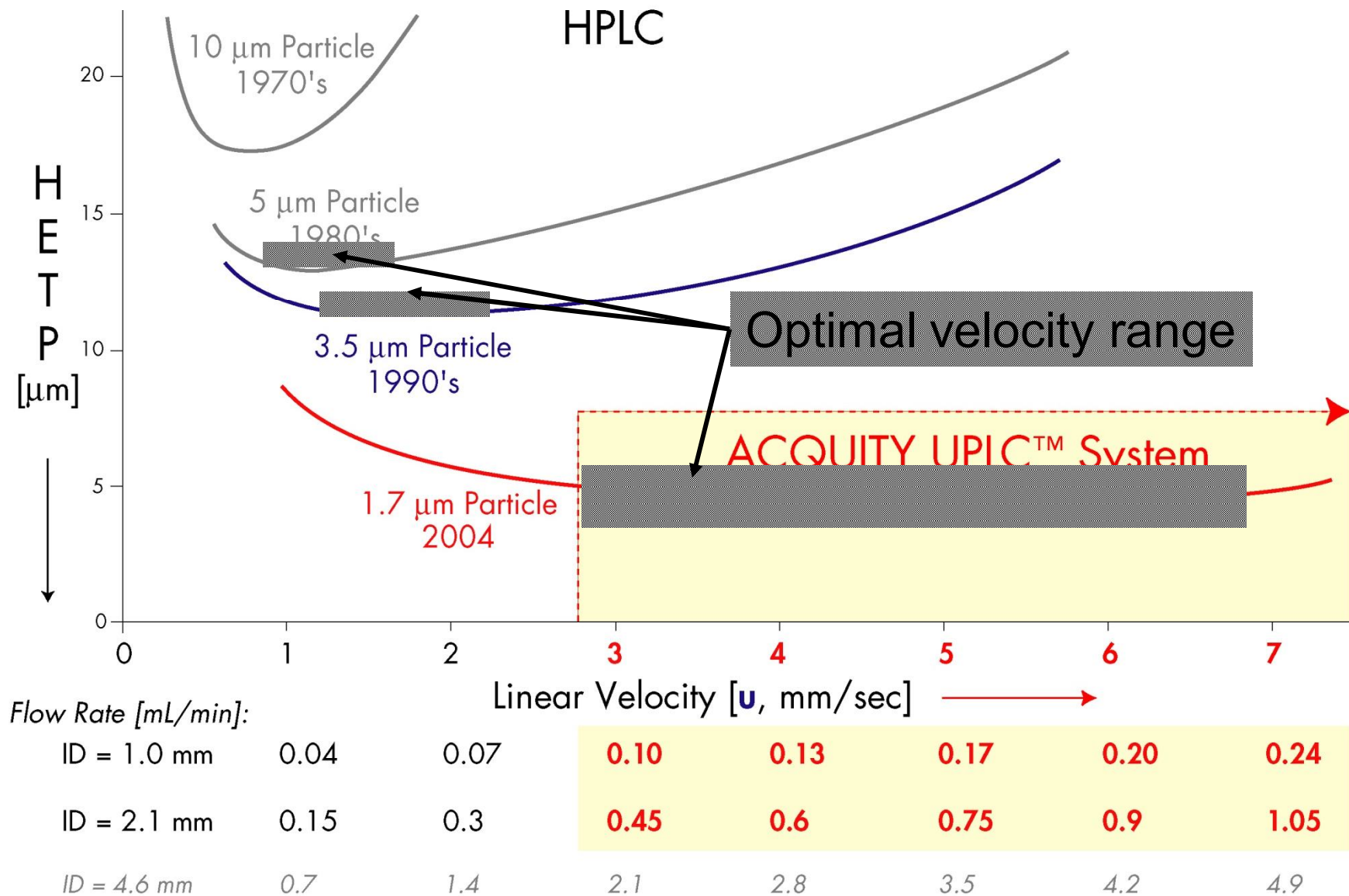
1.7 μm
ACQUITY UPLC® Particles
Optimal Particle Size
Distribution
(leading to lower A term)
For Max Efficiency
at a given Pressure

Chromatography Principles

Mass Transfer / Diffusion



Smaller Particles The Enabler of Productivity



Summary of Key System Attributes

Constant column length

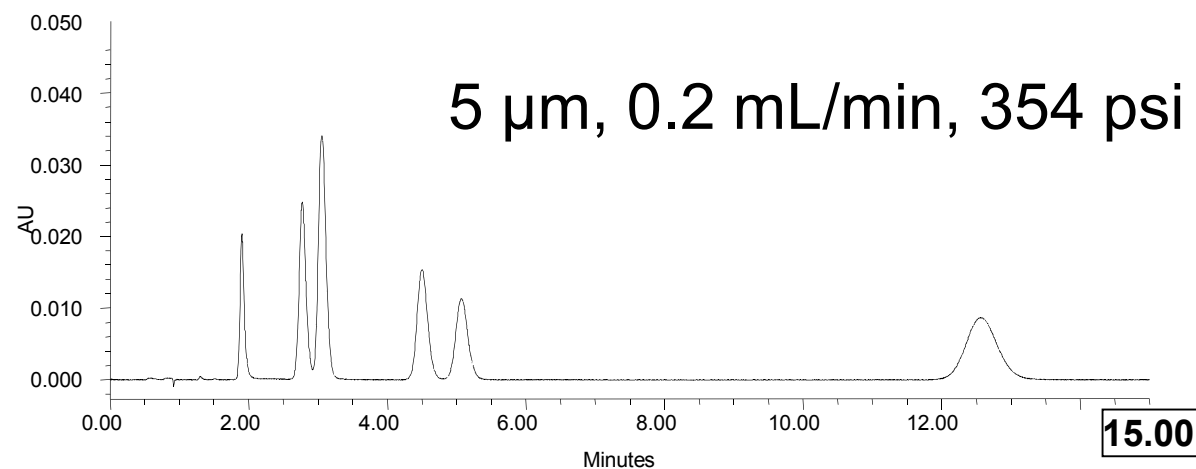
A term + B term + C term

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

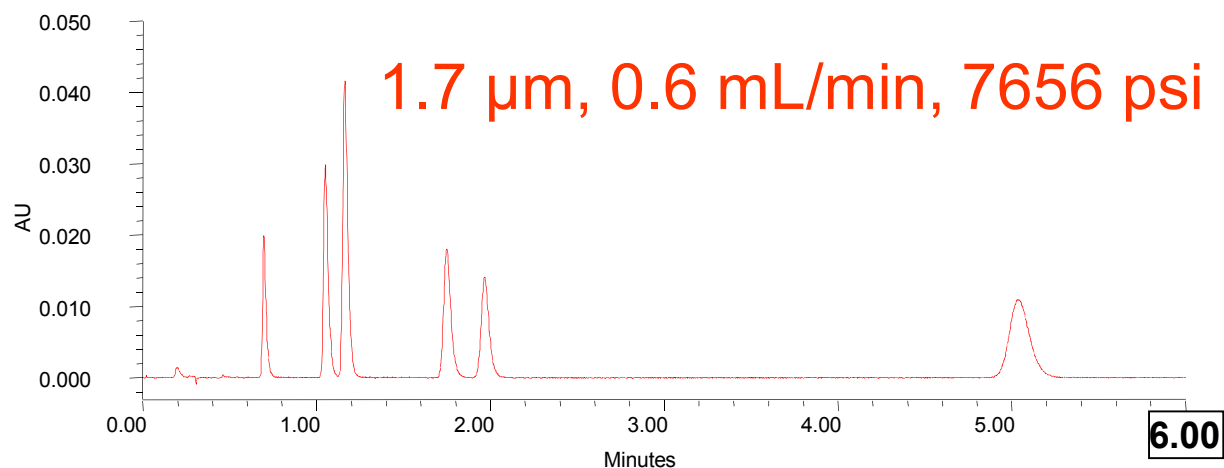
	Resolution Improvement	Speed Improvement	Sensitivity Improvement
5 to 1.7 μm particles	1.7X	3X	1.7X
3 to 1.7 μm particles	1.3X	2X	1.3X

Appendix contains equations in which these values were derived

Fixed Column Length: *More resolution faster*

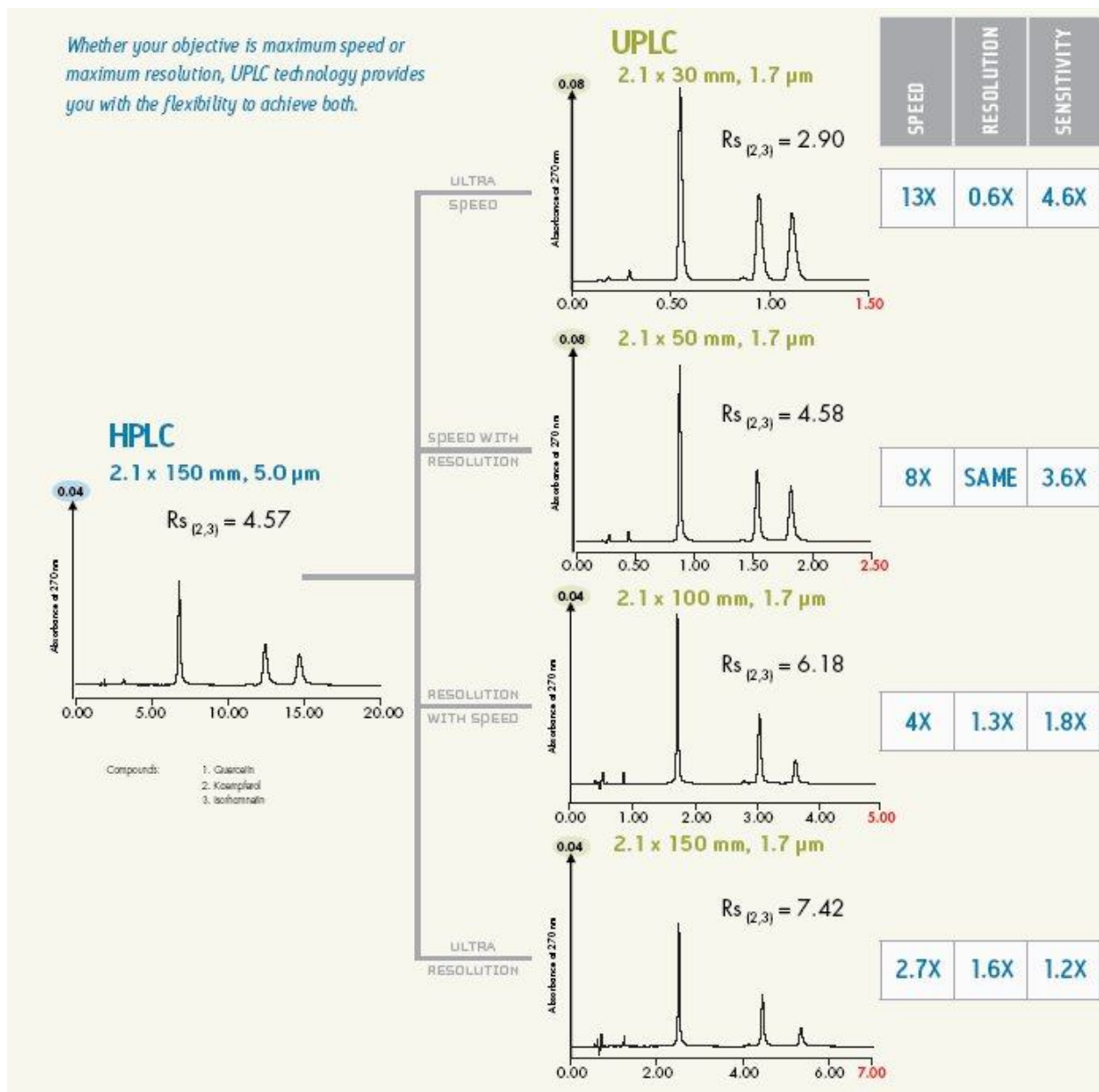


Theory
1.7X Resolution
3X Faster
1.7X Sensitivity
27X Pressure



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

Speed, Resolution, Sensitivity Options



*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

* $dp = 1.7 \mu m$

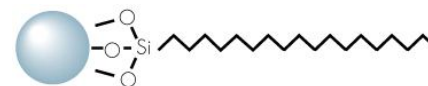
Choosing a UPLC column based upon the relationship between UPLC column length, length to particle size ratio (l/dp), efficiency (N) and separation type.

*From the ACQUITY UPLC Columns Brochure

ACQUITY UPLC® Column Chemistries

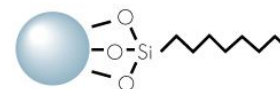
- **BEH C18**

- Trifunctionally Bonded C₁₈
- Proprietary Endcapping
- Widest pH range



- **BEH C8**

- Trifunctionally Bonded C₈
- Proprietary Endcapping
- Widest pH range



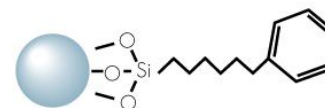
- **BEH Shield RP18**

- Monofunctionally bonded
- Embedded polar group



- **BEH Phenyl**

- Trifunctionally Bonded C₆ Phenyl
- Proprietary Endcapping



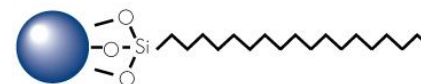
- **BEH HILIC**

- Unbonded BEH particle
- HILIC for very polar bases

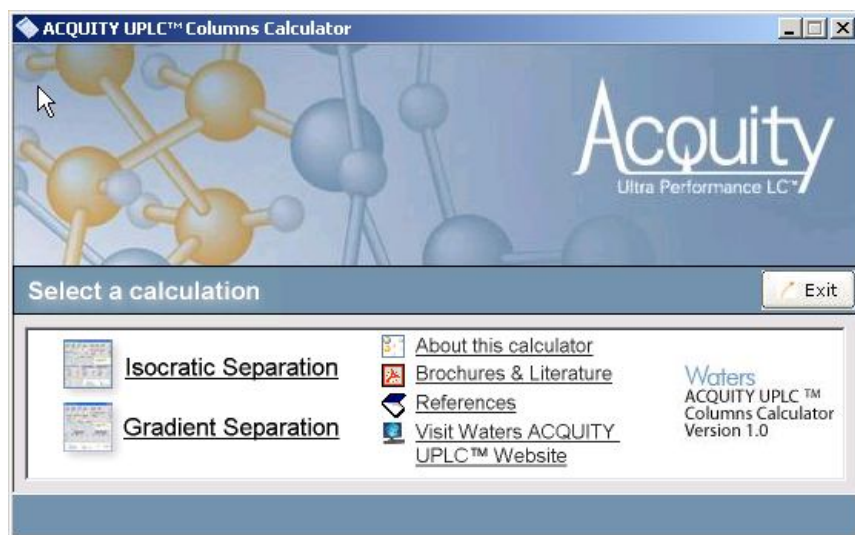


- **HSS T3**

- T3 Bonding & Endcapping
- Superior Polar Compound Retention



Method Transfer Options using the ACQUITY Calculator



The screenshot shows the "Gradient Results" window. The title bar reads "Gradient Results". The window displays "Results With Existing Column" with the following parameters: Peak Capacity: 119, Column Length: 15 cm, Particle Size: 5 µm, Pressure* [PSI]: 1407, Column Diameter: 4.6 mm, MW of Sample: 200. Below this, there are tabs for "Geometrically Scaled UPLC™ Method Choices" and "Optimally Scaled UPLC™ Method Choices", with a "Print" button. The main content area shows two tables of scaled gradient data.

Scaled Gradient 2.1 mm Column (HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
5 cm	2.1	0.313	122	5.1	3619	0.7	View
10 cm	2.1	0.313	178	10.1	7237	1.4	View

Change Flow Calculate HPLC Linear Velocity UPLC™ Linear Velocity

Scaled Gradient 1.0 mm Column (HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
5 cm	1.0	0.071	65	5.1	3619	0.2	View
10 cm	1.0	0.071	116	10.1	7237	0.3	View

Change Flow Calculate HPLC Linear Velocity UPLC™ Linear Velocity

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Method Transfer Options using the ACQUITY Calculator

