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Process Chromatography Basics

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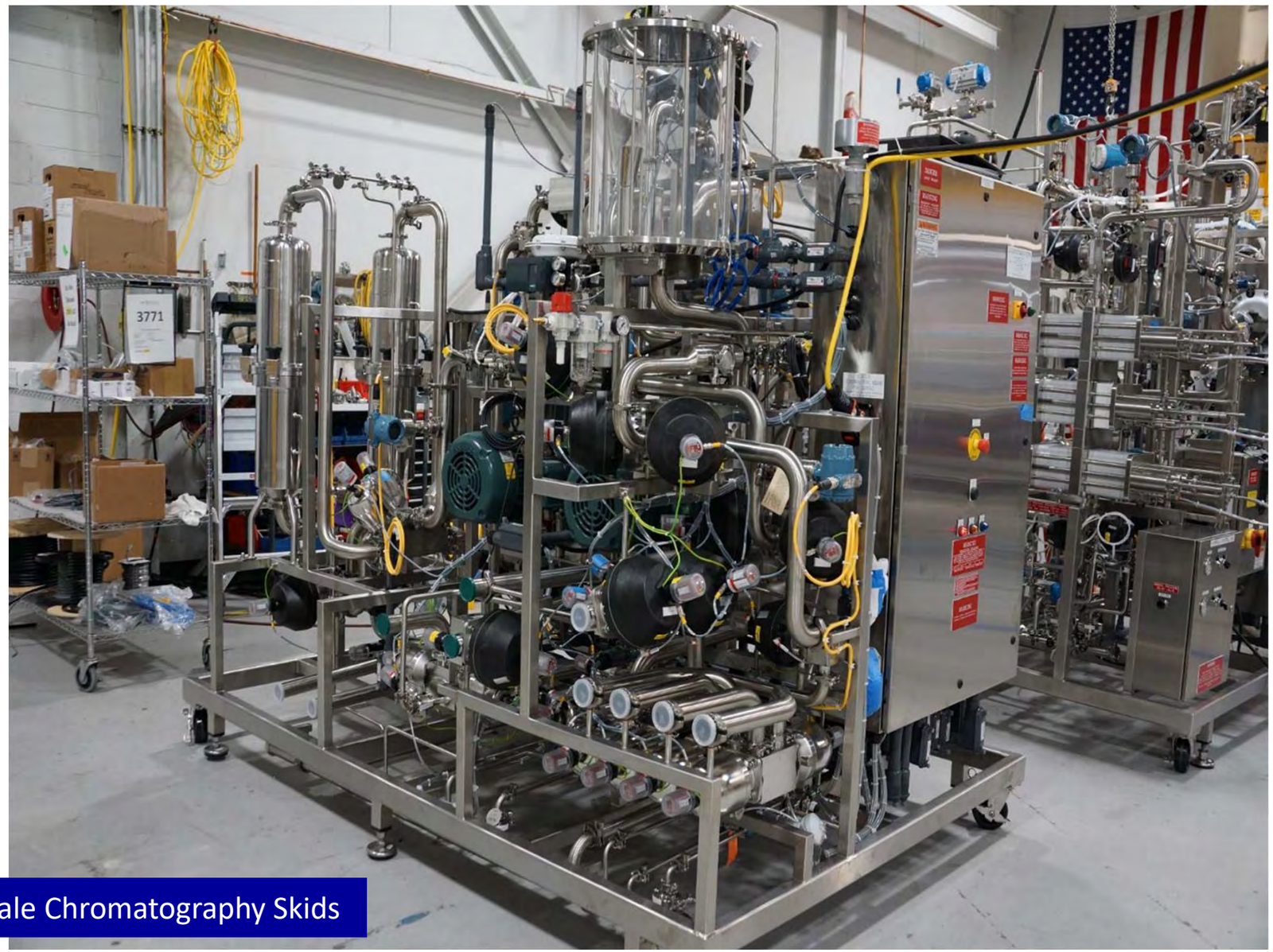

Large Scale Chromatography Skids

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What is Process Chromatography?

- Chromatography – from Greek “color writing”. Process originally developed as means of separating pigments from plant materials.

- Process Chromatography is a technique for separating the components of a mixture by taking advantage of the differences in their mobility as they pass through a column of packed media.

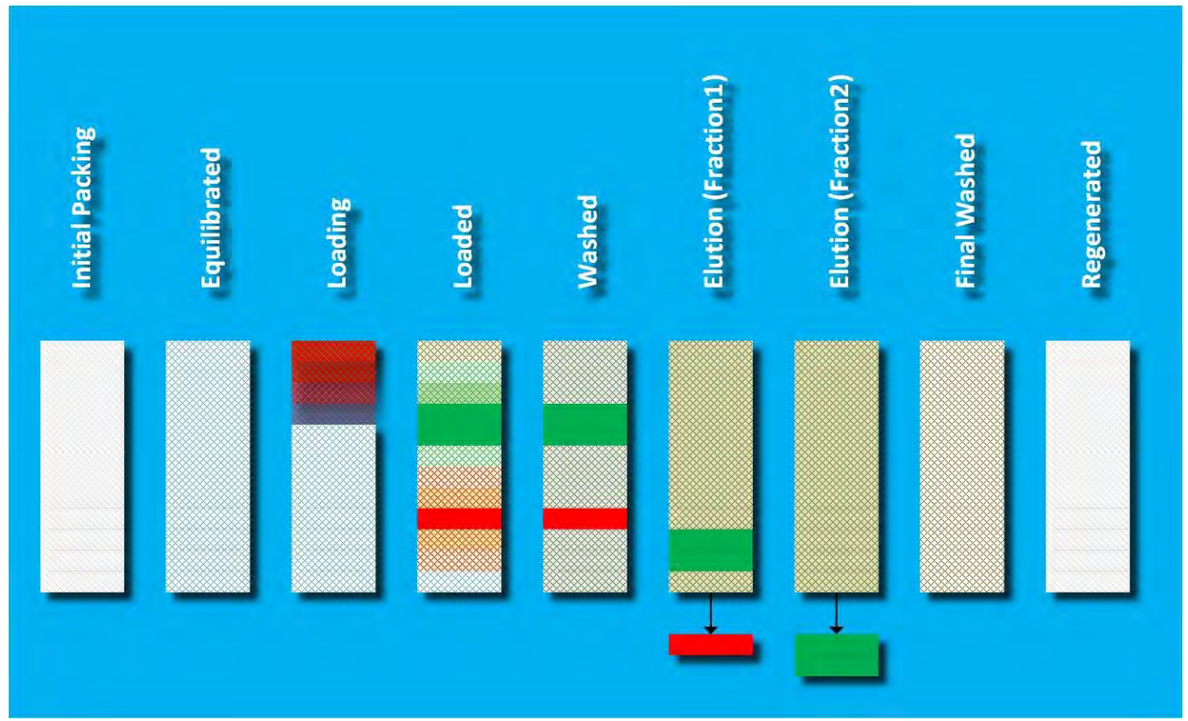
- Today, chromatography plays an essential role in downstream pharmaceutical manufacturing, providing a means of concentrating and purifying target molecules.

Chromatography Separation Methods

- Size Exclusion Chromatography (SEC)
  - Separates molecules based on their relative size - filtration through porous beads
- Ion Exchange Chromatography (IEX)
  - Separates molecules based on their relative ionic attraction to positively or negatively charged sites on the surface of porous media
- Hydrophobic Interaction Chromatography (HIC)
  - Separates molecules based on their relative hydrophobic or hydrophilic attraction to sites on the surface of porous media
- Affinity Chromatography
  - Separates molecules based on specific binding interaction between them and their partner size
    - Antibody / antigen
    - Enzyme / substrate
    - Enzyme / inhibitor
- Multimodal or Mixed Mode Chromatography
  - Separates molecules using more than one of the techniques above

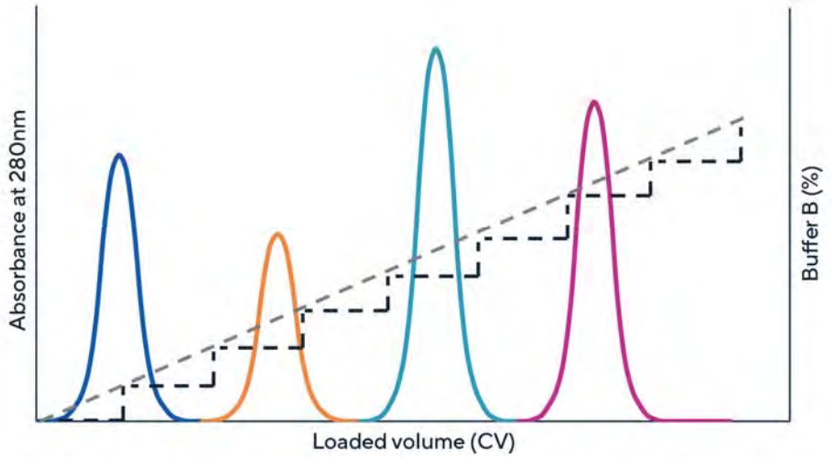
Typical Workflow for LPLC

- Column Equilibration
  - Introduction of a buffer (5-10 BV) to suitably prepare the media for loading
- (Sample) Loading
  - Introduction of the feed mixture into the column
- Washing
  - Introduction of a buffer that removes unbound molecules from the column
- (Sample) Elution
  - Introduction of a buffer that releases bound molecules from the media
- Final Column Washing
  - Introduction of a buffer that removes remaining elution buffer and trace amounts of loaded molecules
- Regeneration
  - Introduction of a buffer or series of buffers to place the media back into a suitable state for the next chromatography cycle



Continuous chromatography offers two benefits to this process – full utilization of column capacity and, having a column available for loading at all times, while another column(s) is being eluted or prepared for loading

Elution

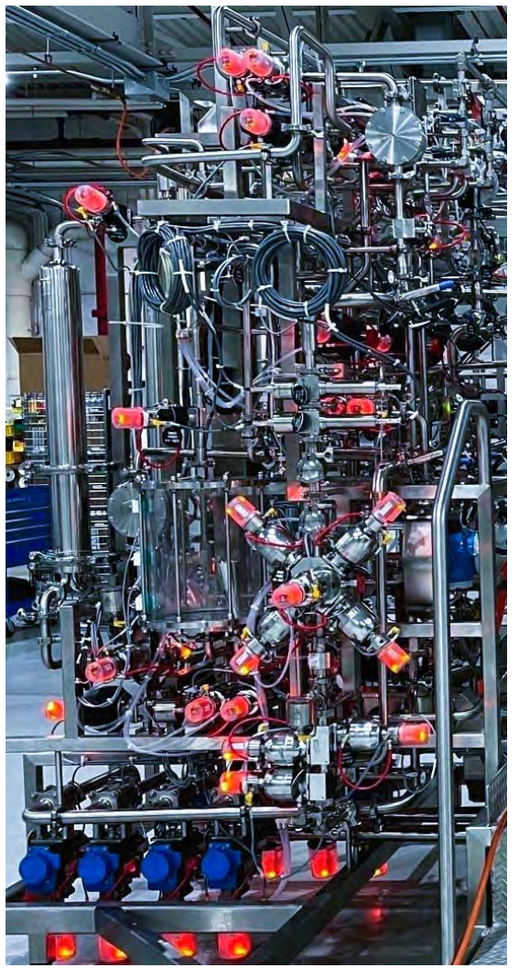


- Isocratic Elution
  - Elution performed with a single buffer concentration
- Gradient Elution
  - Elution performed with varied buffer concentration*
    - ❖ Step Gradient
      - A gradient that is achieved by changing the elution buffer concentration in a step fashion at distinct intervals
    - ❖ Continuous Gradient
      - Essentially a step gradient where the change intervals are so small that the concentration change appears to be continuous

** Gradient concentrations will always be limited on each end by the minimum flow rate achievable by the pumps*

Critical Concepts for Chromatography

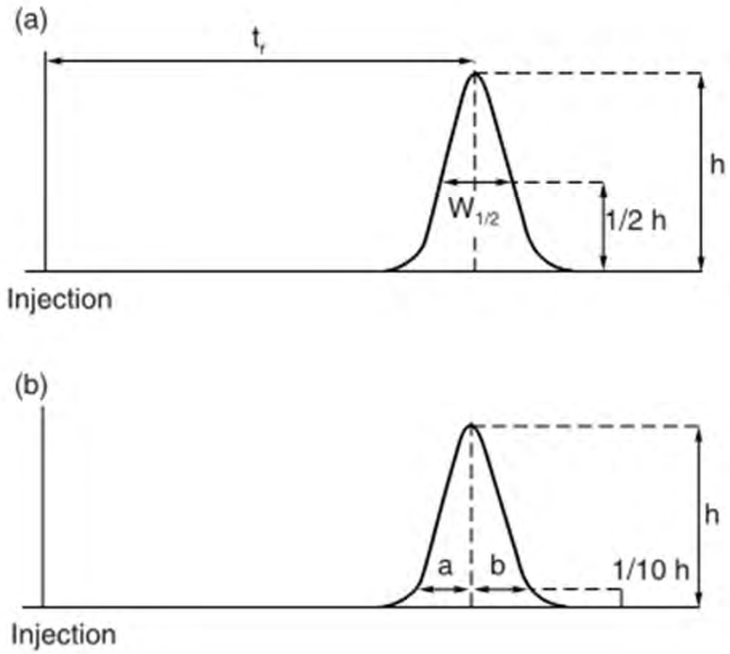
- Limit Hold-Up Volume
  - Reduces product loss and buffer usage
- Remove Air Bubbles / Monitor for the presence of air
  - Bubbles will get caught in the media and block active sites causing efficiency losses
- Flow & Volume Control
  - Column flux rates and bed volumes can be critical to the kinetics of loading, elution and regeneration
- HETP (Height Equivalent of Theoretical Plate)
  - This is a method of baselining column performance by periodically introducing a known sample to a prepared column to verify its efficiency against a properly packed and prepared column (see detailed slide)
- Cleanability / Sanitizability & Lay-up
  - CIP paths need to be well thought out to ensure proper removal of product materials
  - Units will typically be chemically sanitized due to media temperature limits
  - Units will often be “laid-up” (stored) in a solution similar to (lower concentration version) the sanitization solution.
- Drainability
  - System should be gravity drainable or process air/N2 blow out considered for small tube sizes



Height Equivalent of Theoretical Plate (HETP)

Evaluates a column's performance by monitoring the time characteristics associated with flow of a known buffer through the column

1. Retention (t_r): Time to max peak height
2. Curve Area (A): Area under curve above baseline
3. Peak Height (h) : Maximum height above baseline
4. Width at base (W): Total peak width
5. Width at half height ($W_{1/2}$) : Peak width at ½ peak height
6. Plate height (HETP) : = $\frac{L}{5.54\left(\frac{t_r}{W_{1/2}}\right)^2}$ where L = Bed Height (cm)
7. Asymmetry endpoints (t_a & t_b): Time values associated with the points that intersect the peak curve at 1/10 of the overall peak height, where "a" is on the increasing slope of the curve and "b" on the decreasing slope.
8. Asymmetry (A_f): = $\left(\frac{t_b - t_r}{t_r - t_a}\right)$



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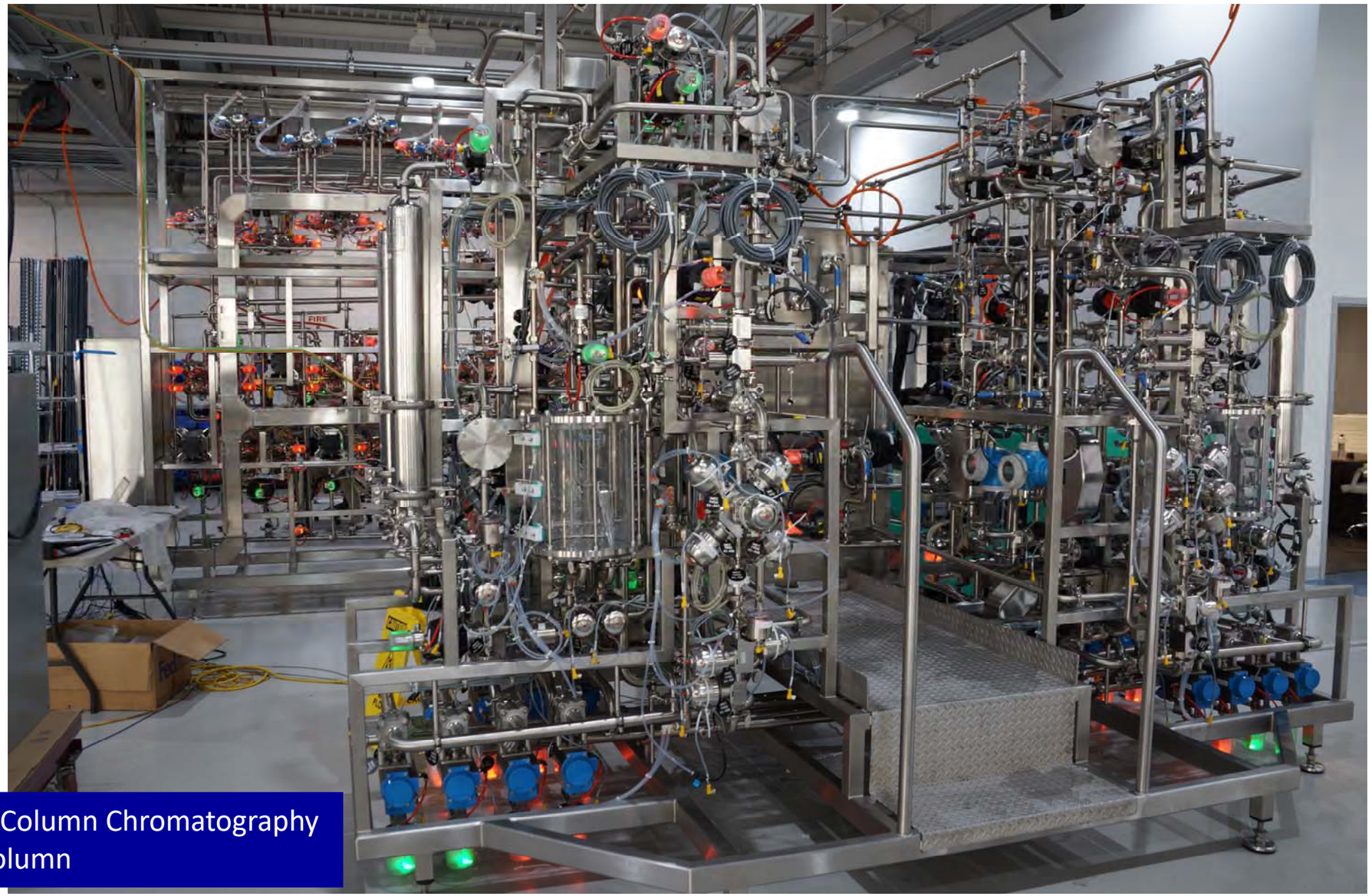

Sequential Multi-Column Chromatography Skid (SMCC) 4- Column

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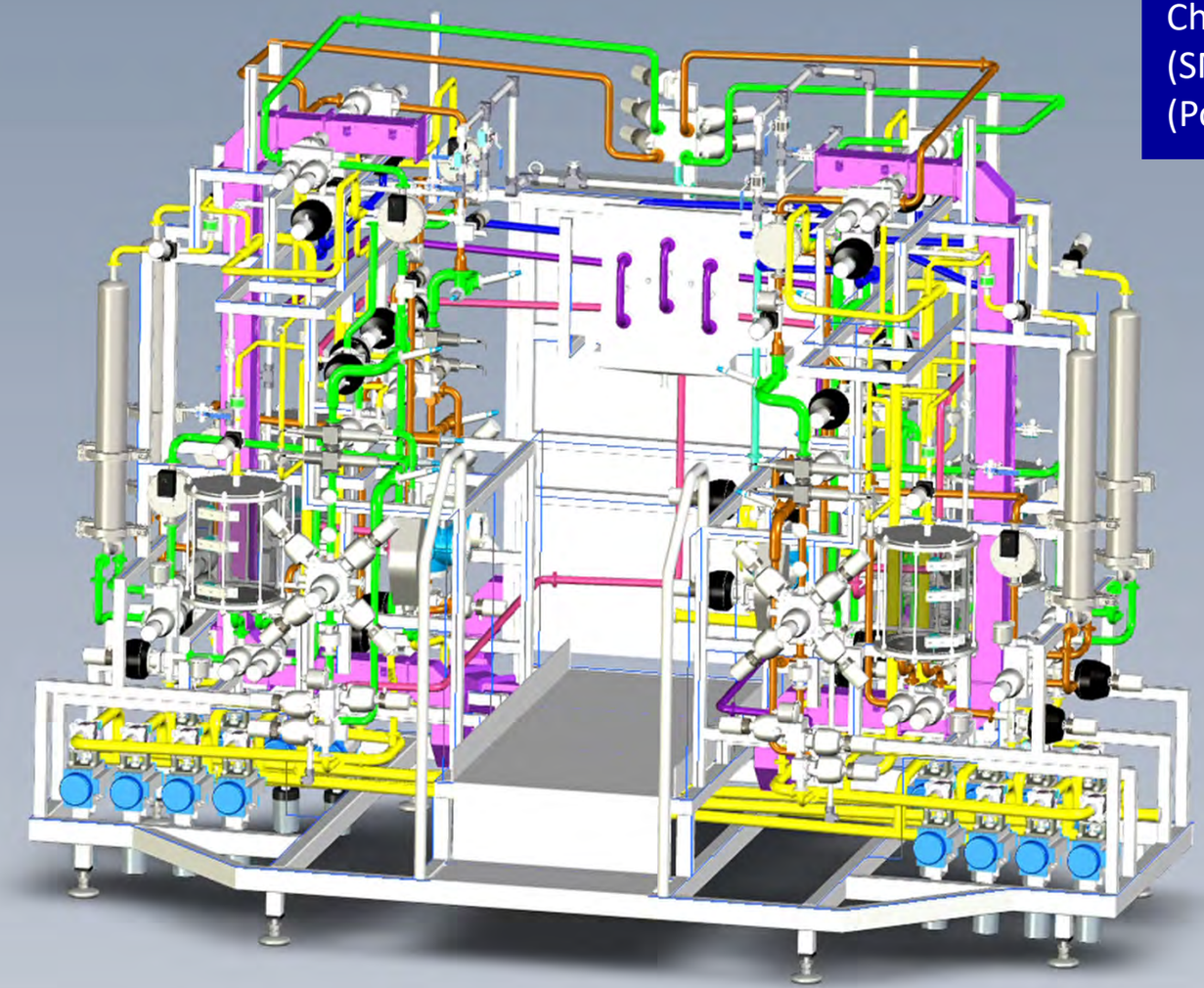
Sequential Multi-Column Chromatography Skid (SMCC) 4- Column (Portsmouth, NH)



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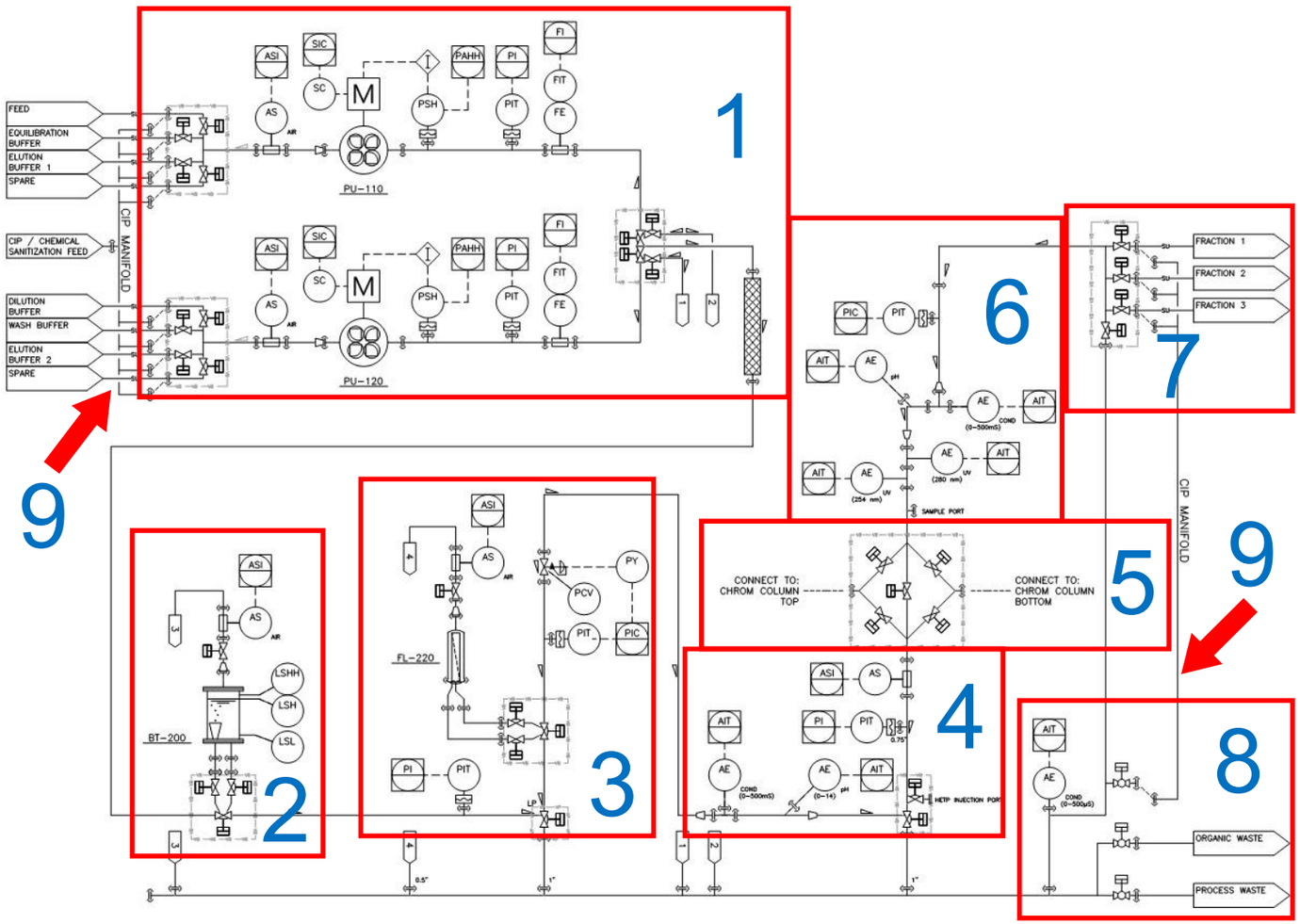

Typical LPLC P&ID

1. Feed Pumps / Dilution / Blending
2. Bubble Trap
3. Filtration & Backpressure Control
4. Pre-Column Analytics
5. Column Distribution Valve
   - "Chrom Valve"
6. Post-Column Analytics
7. Fractionation Valves
8. Rinse Conductivity & Waste Segregation Valves
9. CIP / Sanitization Manifolds

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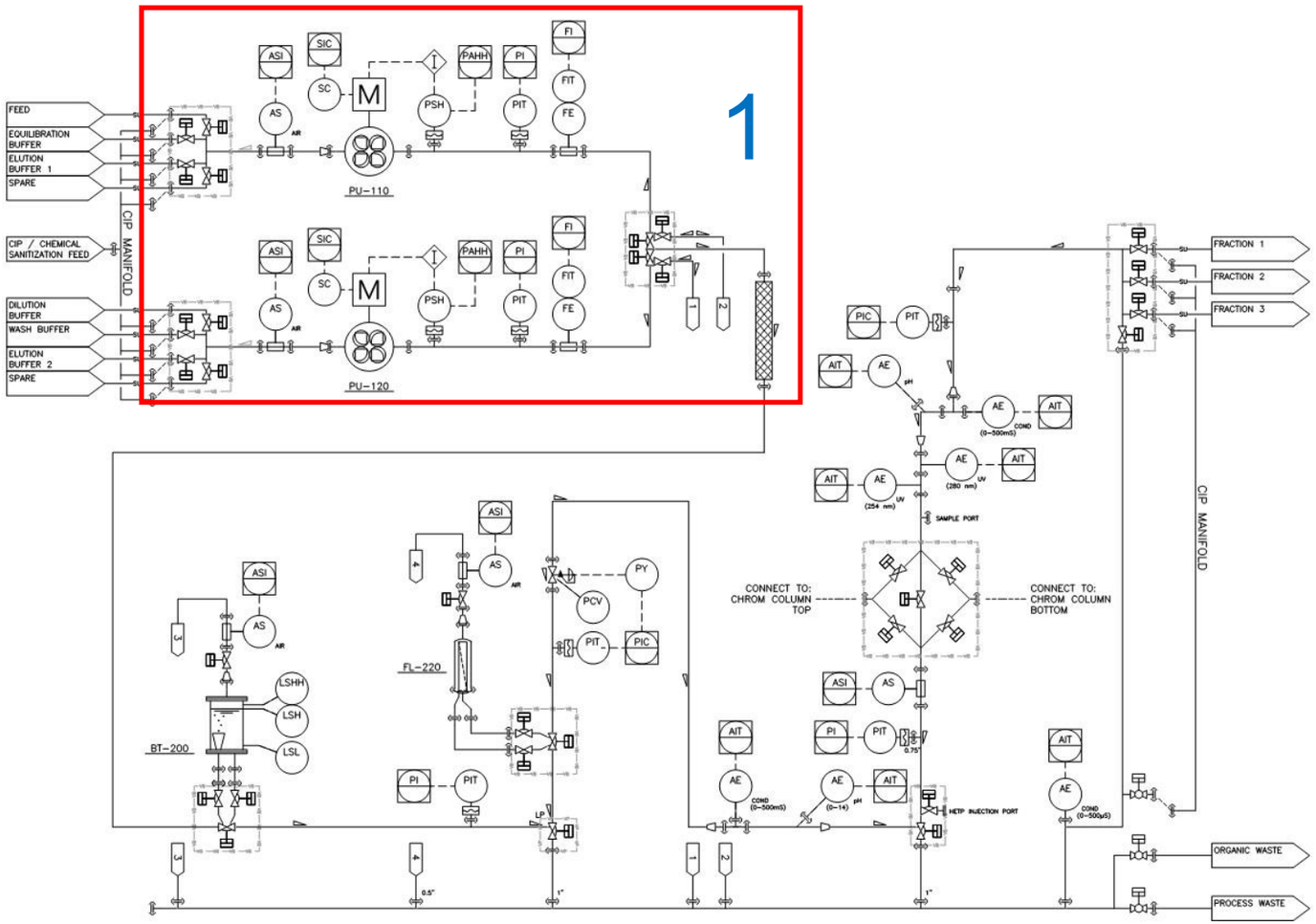

1. Feed Pumps / Dilution / Blending

- Multiport Drainable Feed Valves (quantity of feed ports is customizable)
- Feed Air Sensors
- High Precision Metering Pumps with feedback flow controls
- Isocratic & Gradient control routines
- Hardwired overpressure interlock
- Priming / Displacement Valves
- Static Mixer

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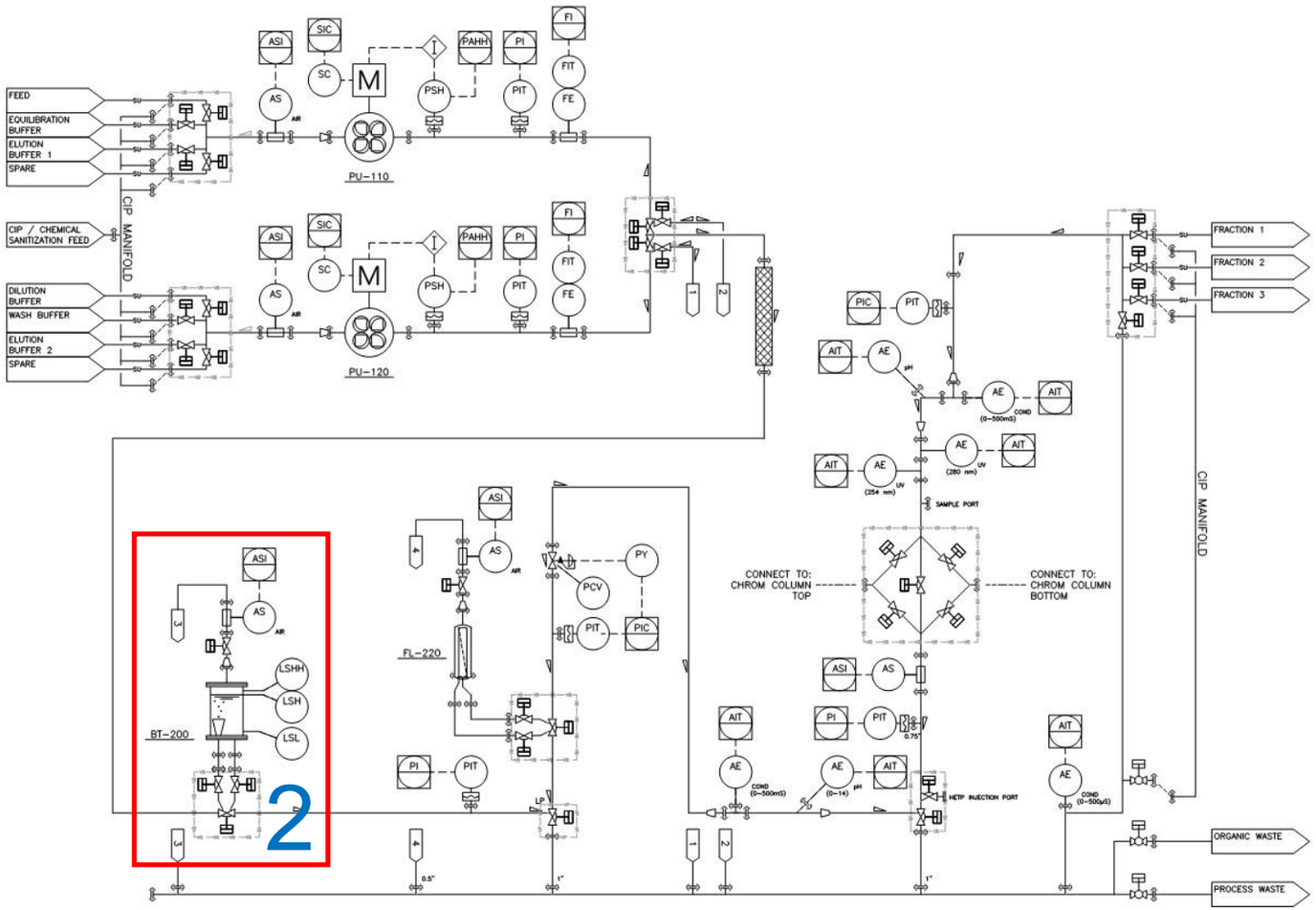

2. Bubble Trap

- Diverter Valves with By-pass
- Bubble Trap Level Monitoring
- Trap overflow for CIP & Chemical Sanitization
- Air Sensor on Trap Vent

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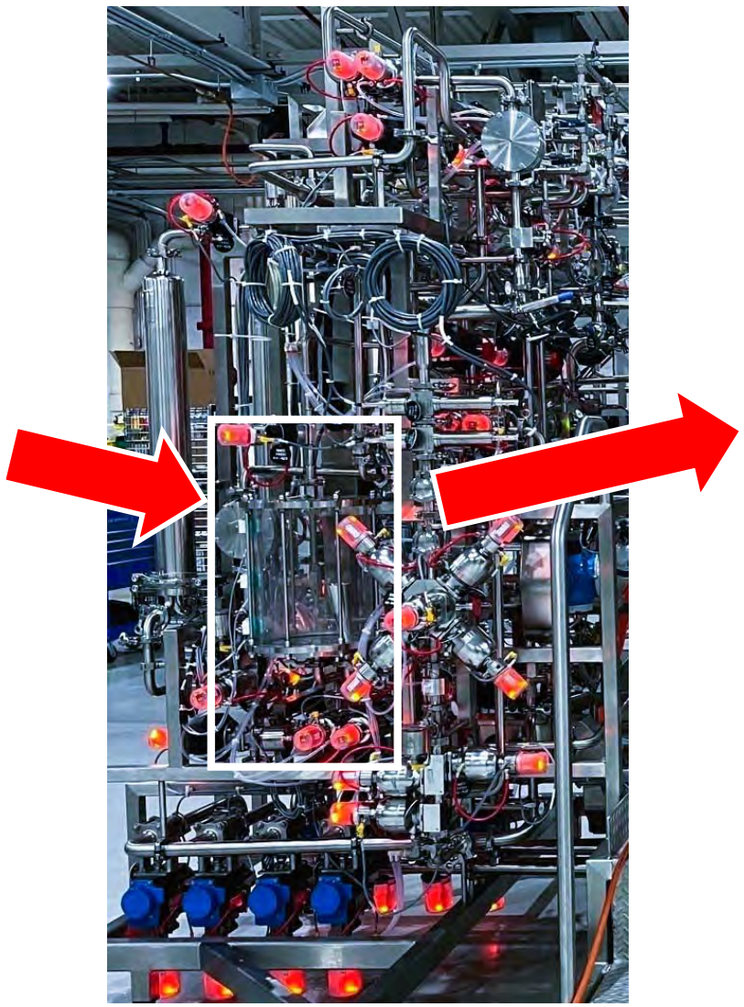

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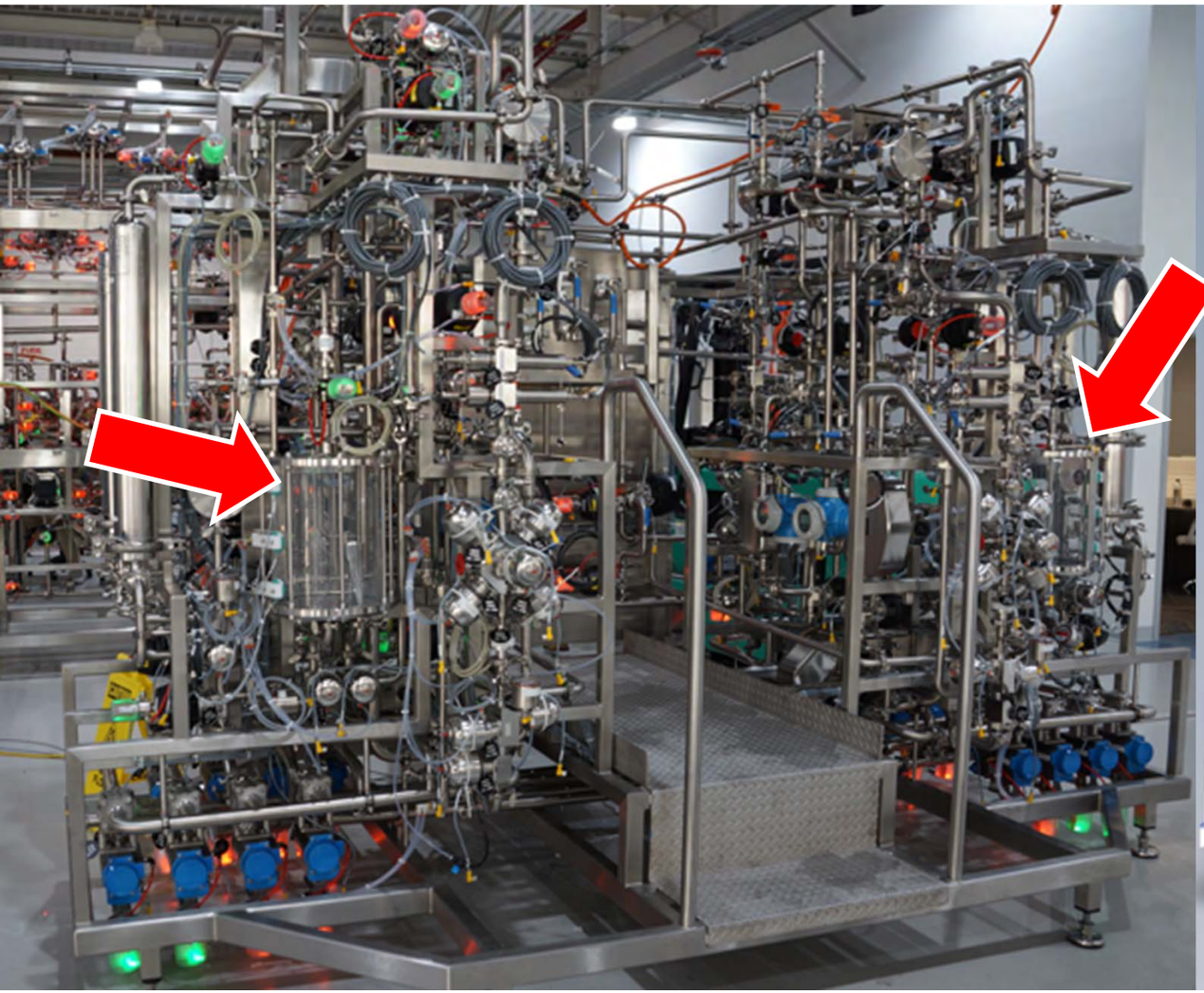

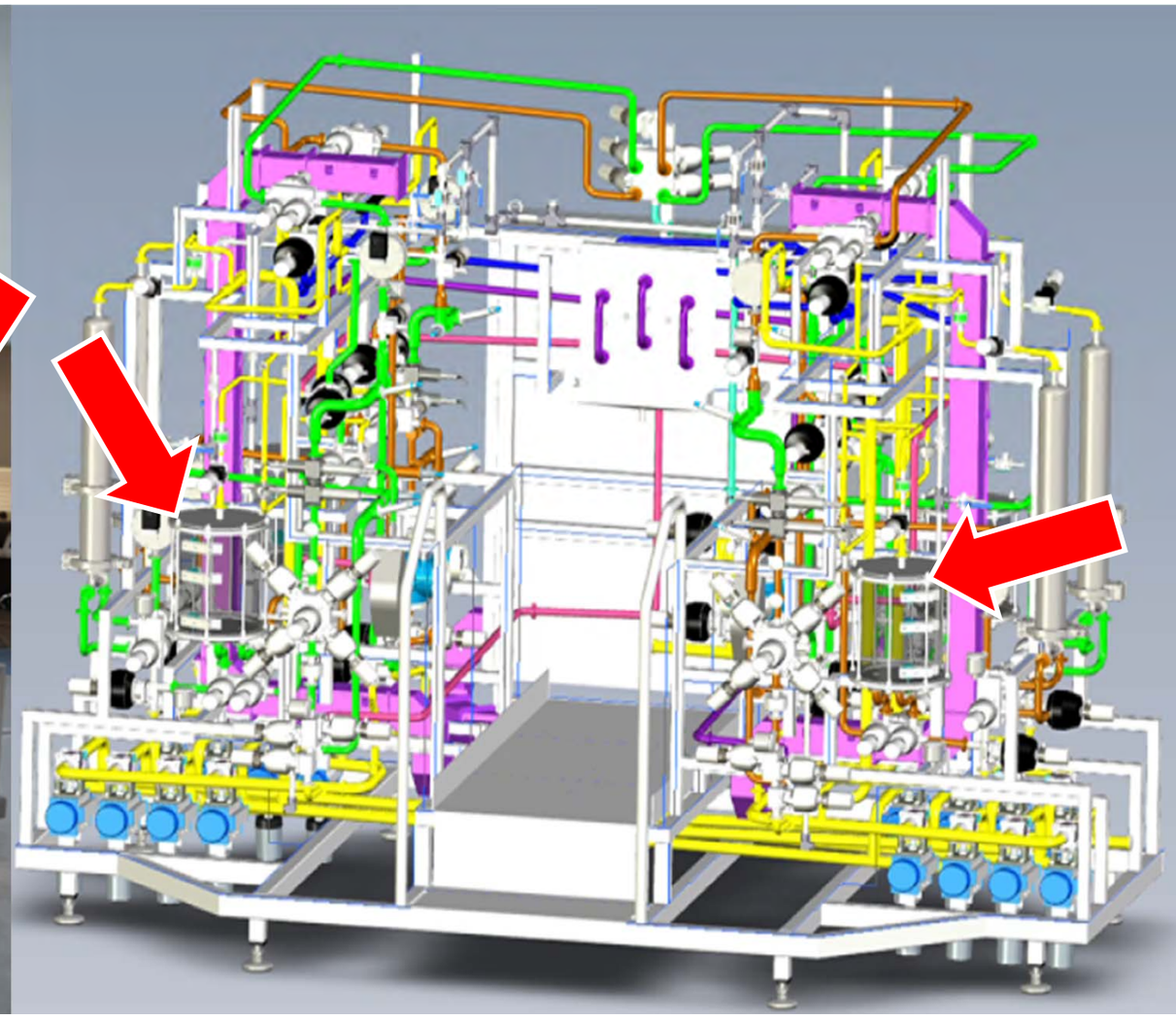

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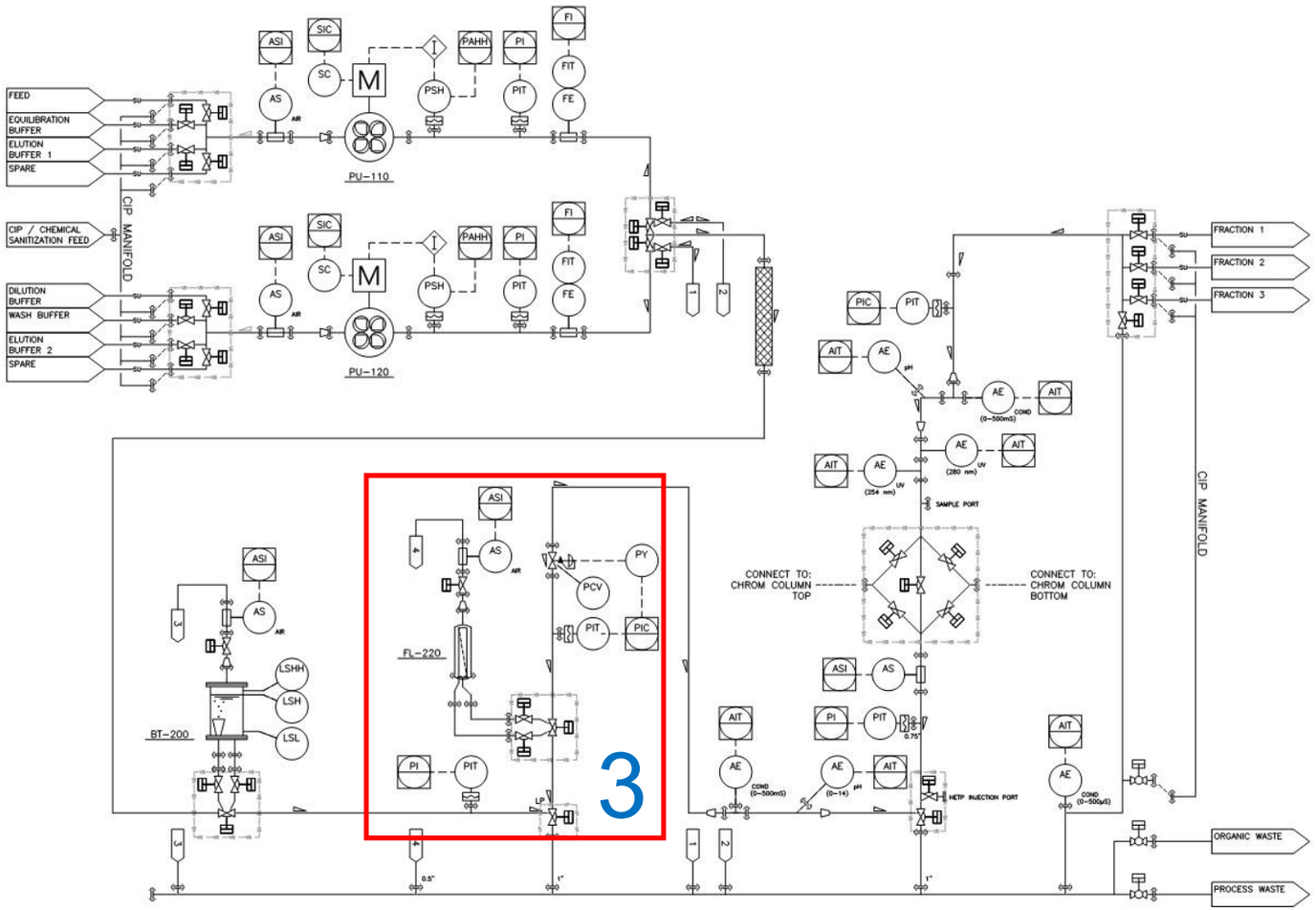

3. Filtration & Backpressure Control

- Feed & Discharge Pressure Transmitters
- Diverter Valves with By-pass
- Filter overflow for CIP & Chemical Sanitization
- Air Sensor on Filter Vent
- Feed backpressure control valve (setpoint controllable per phase step or recipe)

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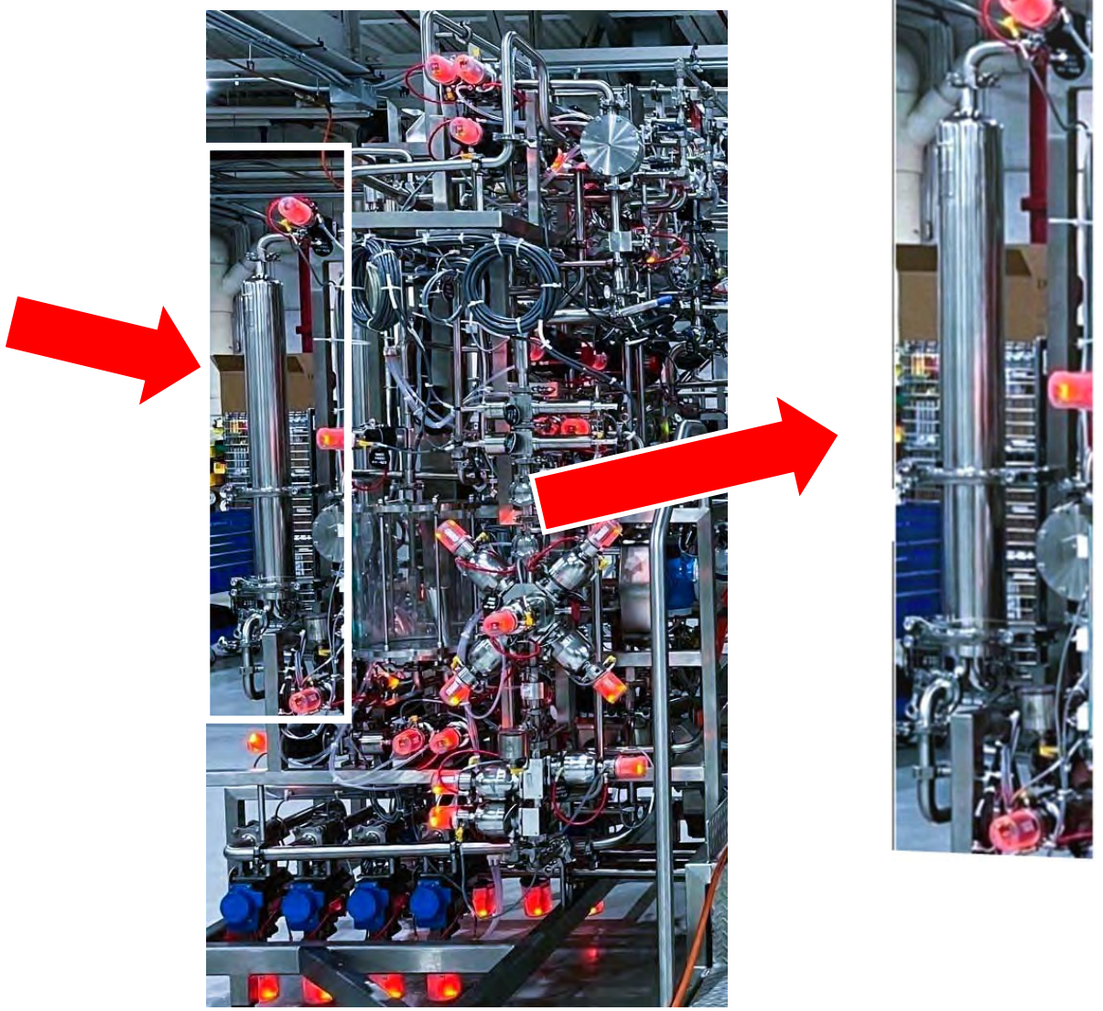

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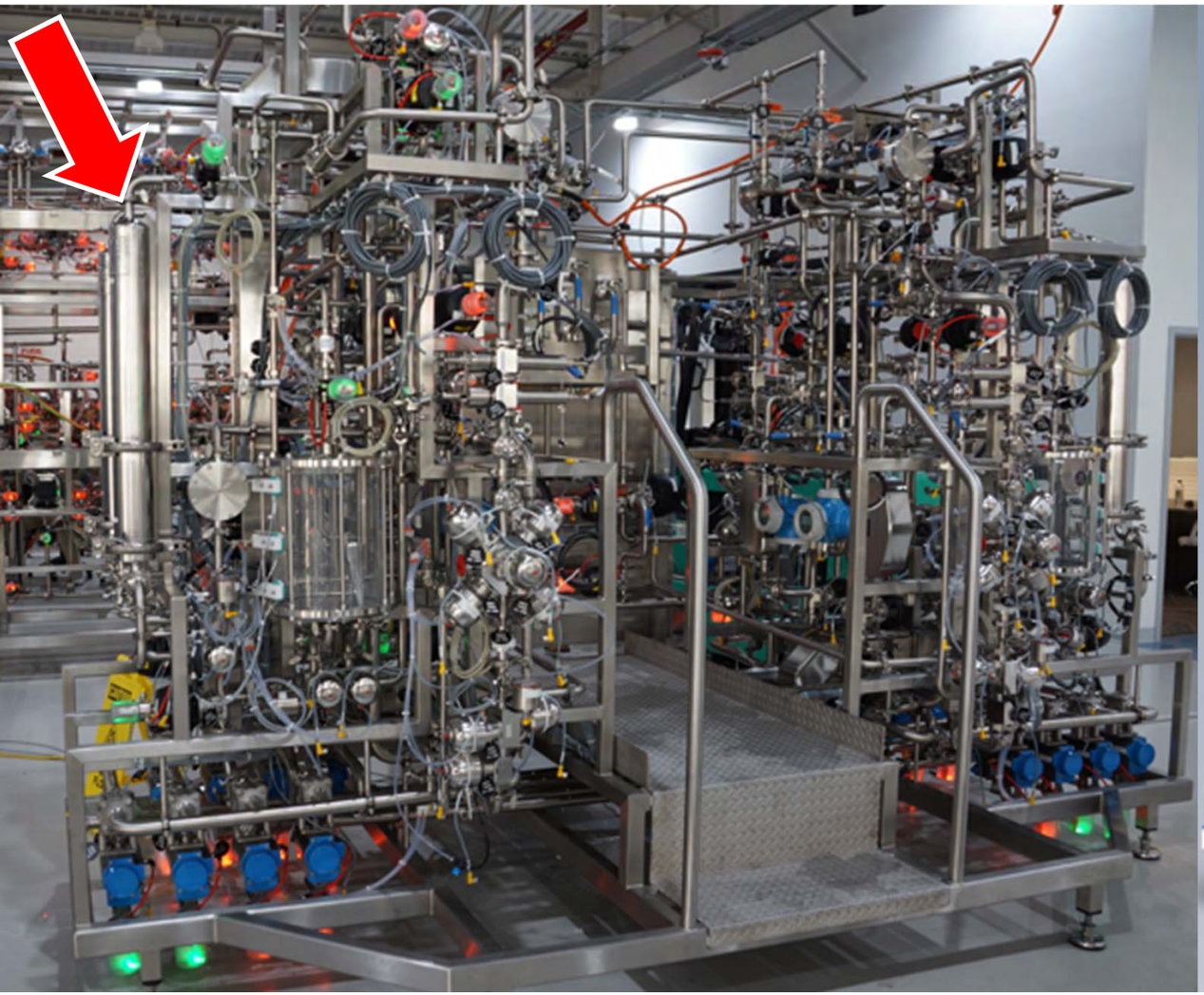

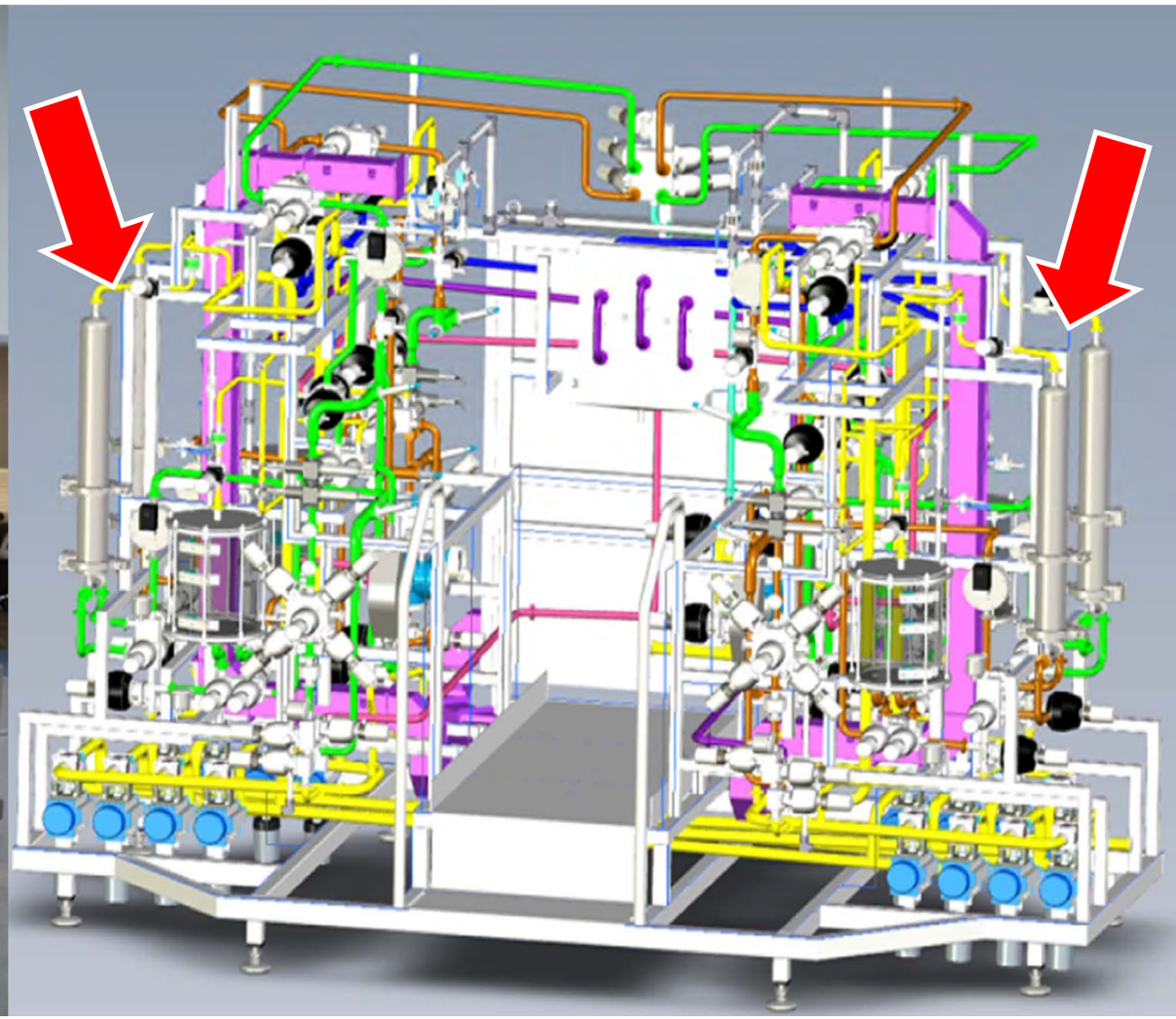

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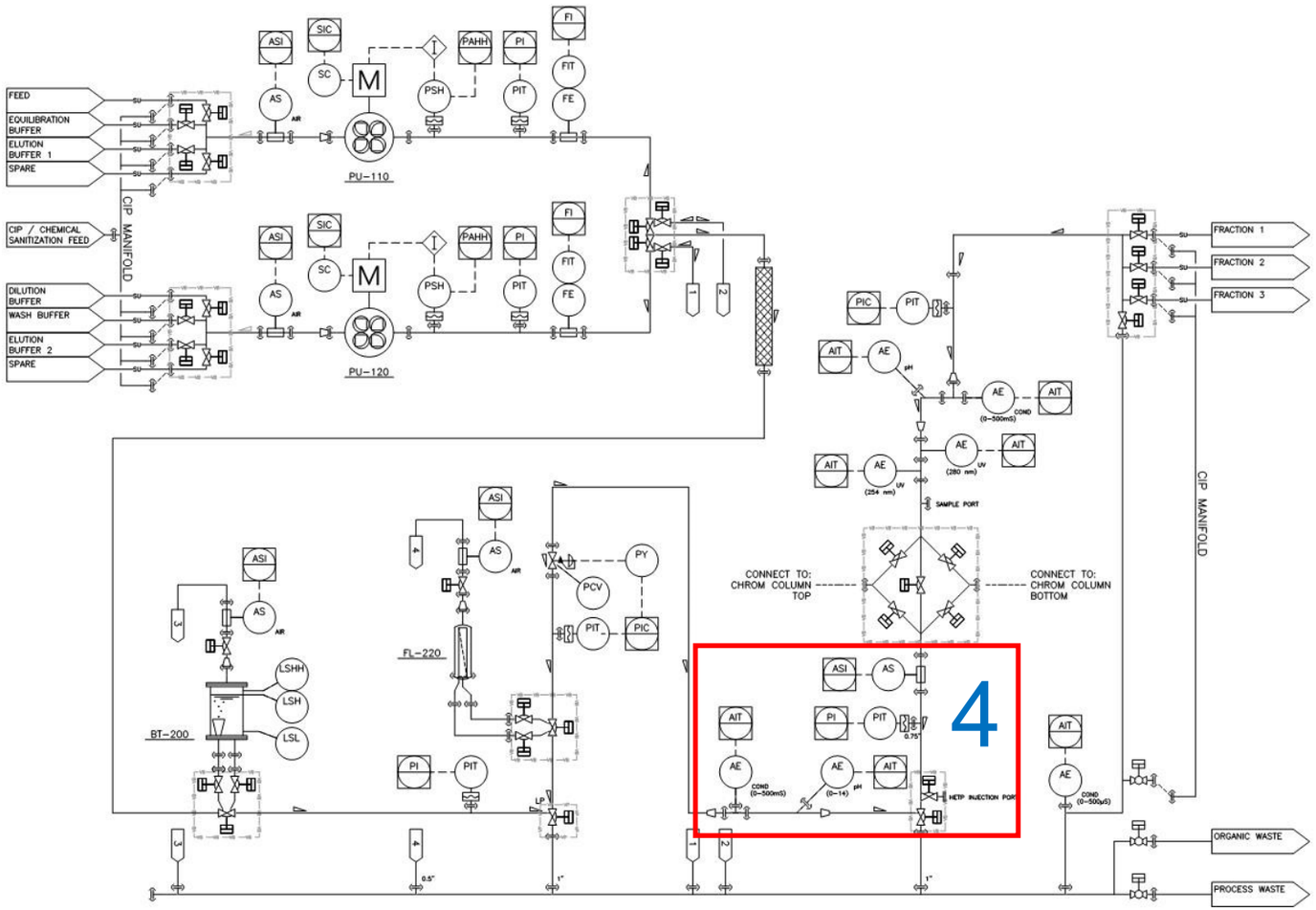
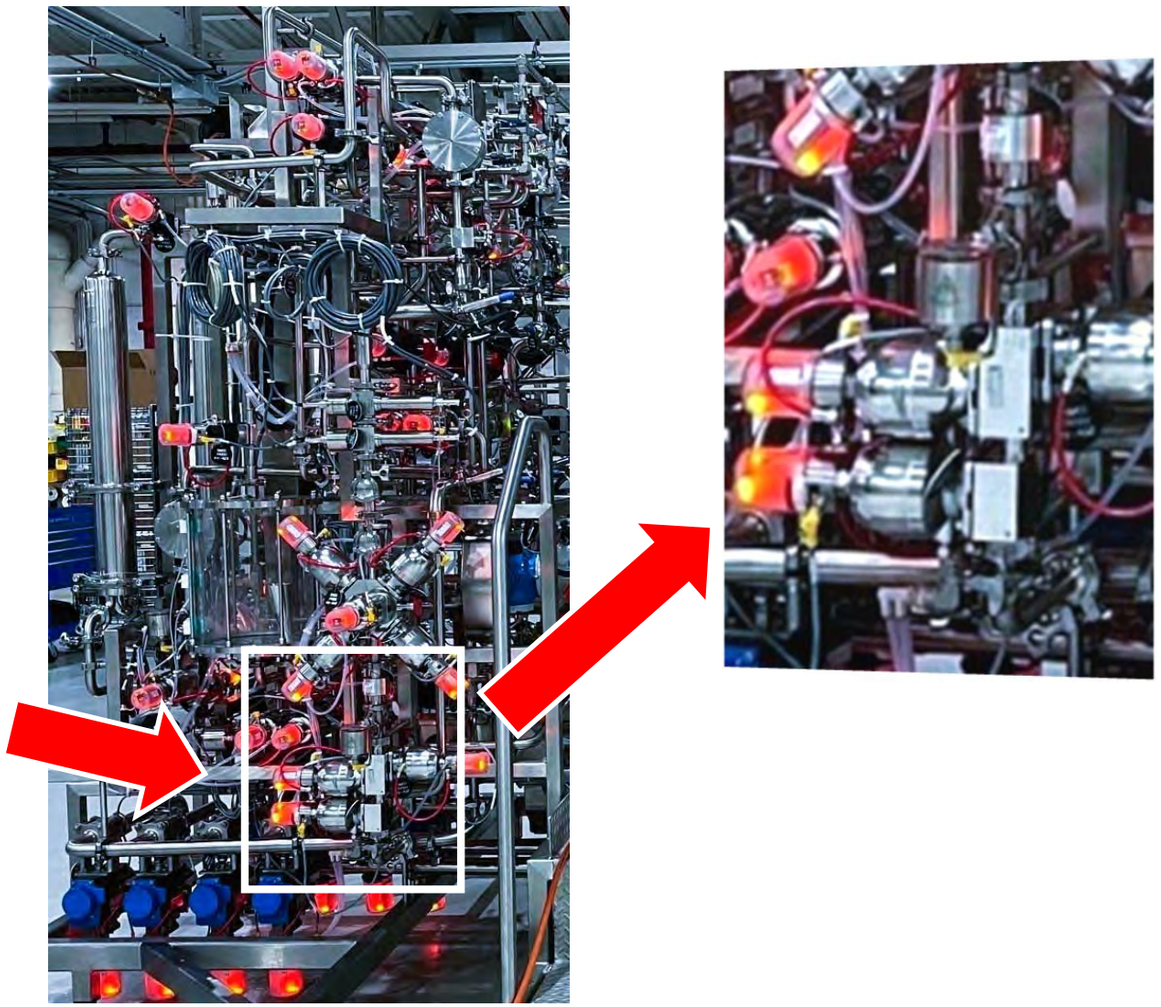


4. Pre-Column Analytic Options

- Pre-Column Conductivity
- Pre-Column pH
- Pre-Column UV Sensor (not shown)
- Pre-Column Pressure Transmitter
- Pre-Column Air Sensor
- HETP Injection Point Valve



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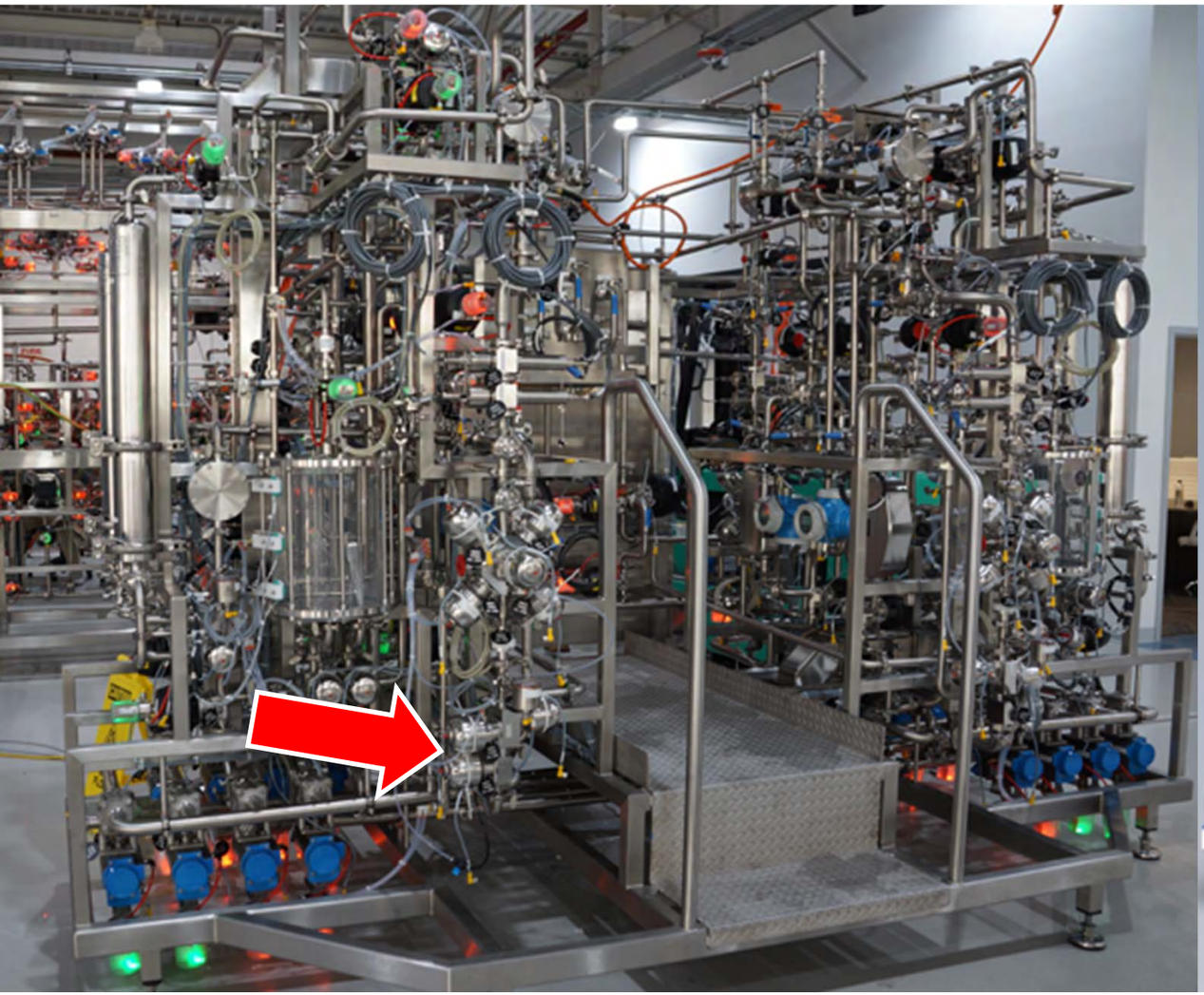

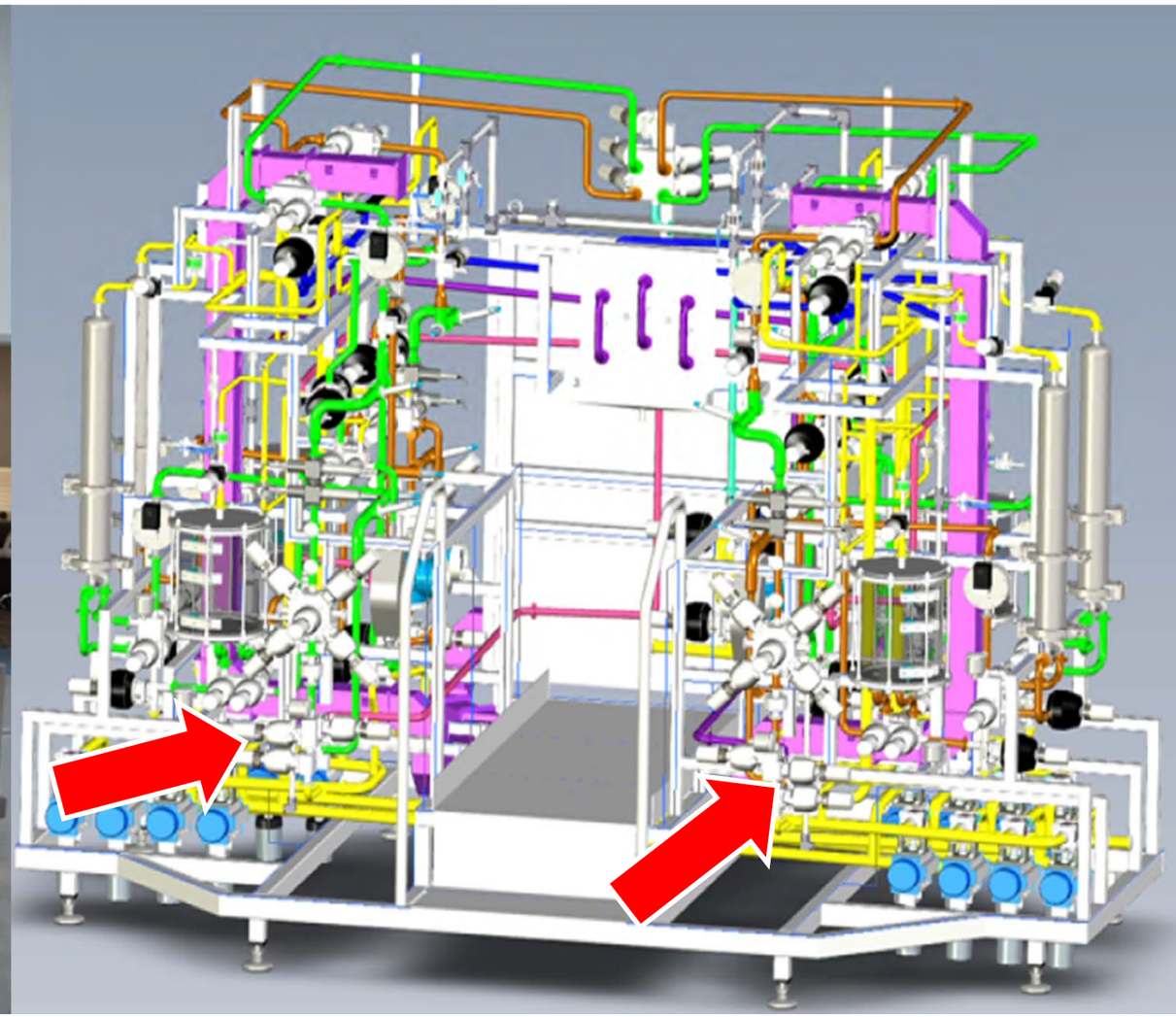

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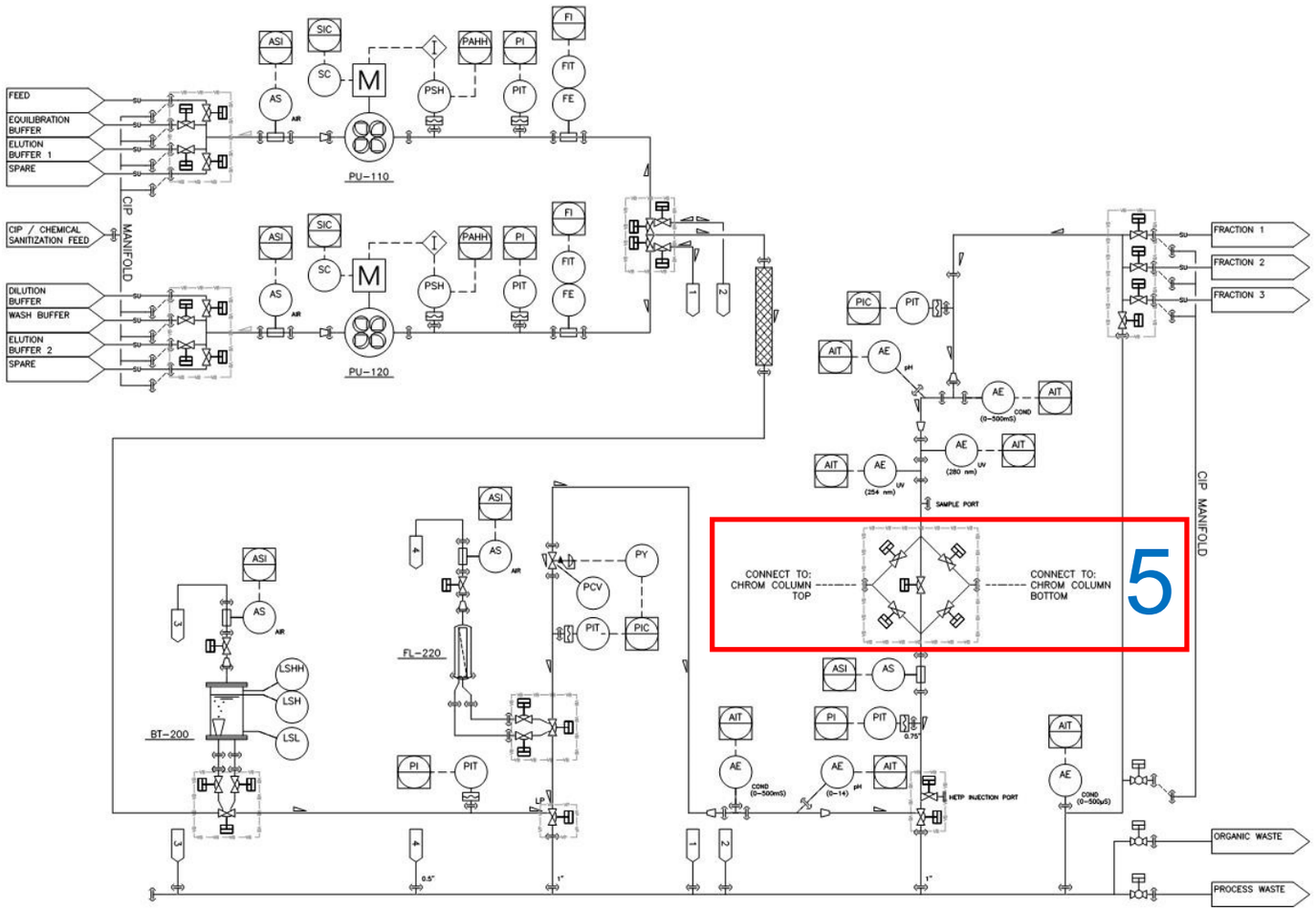
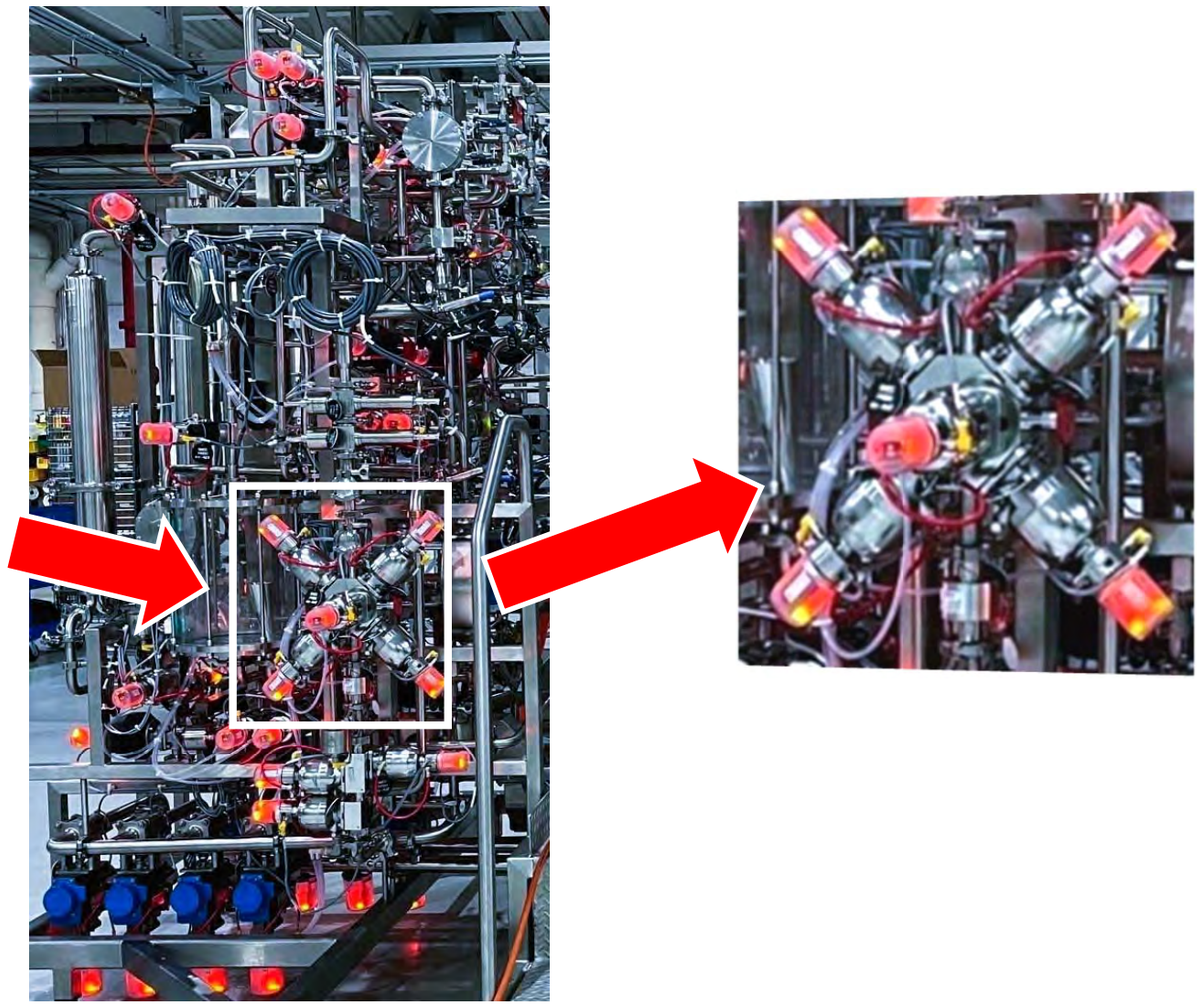


5. Column Distribution Valve

- Column By-pass
- Column Downflow Capability
- Column Upflow Capability



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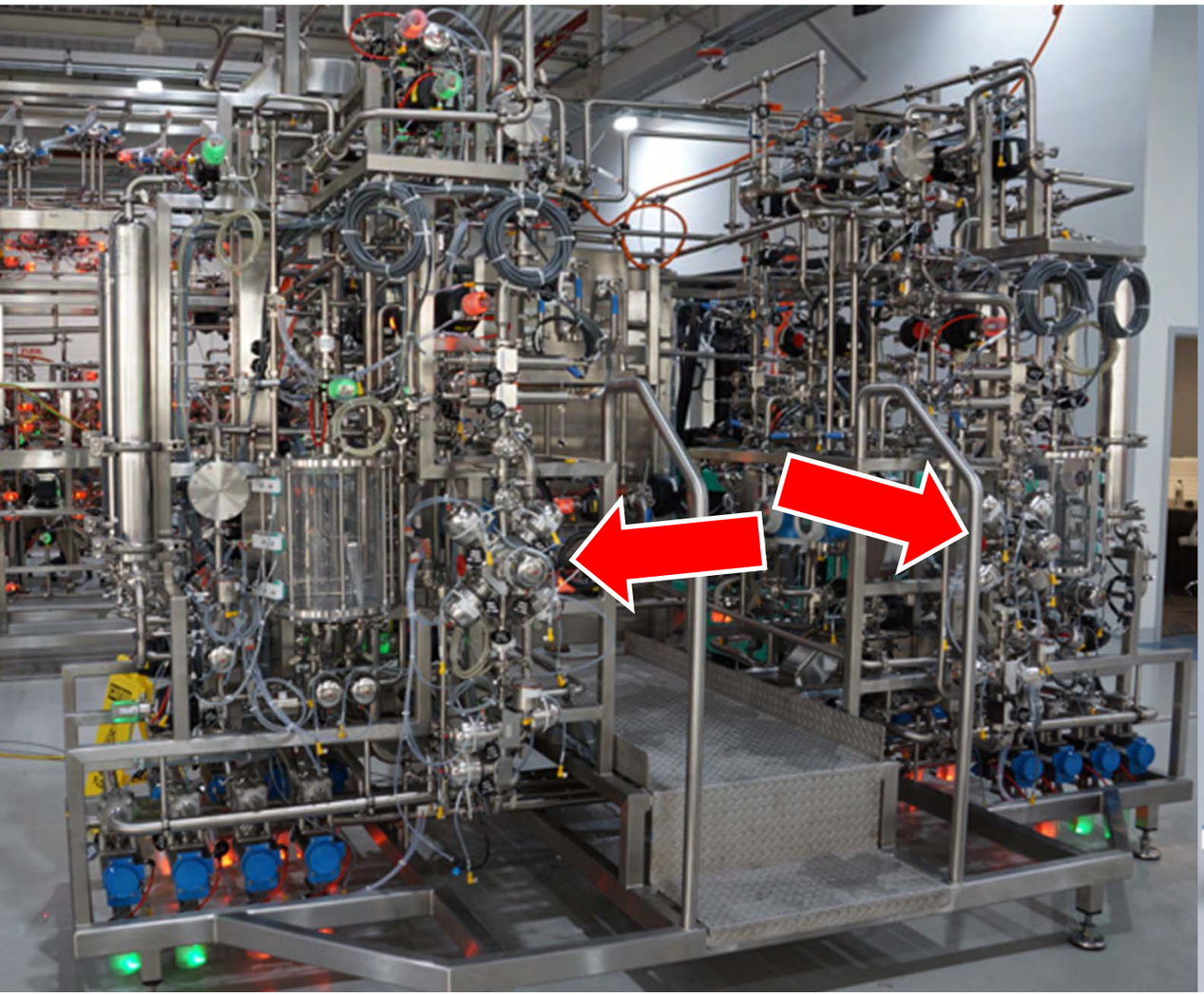

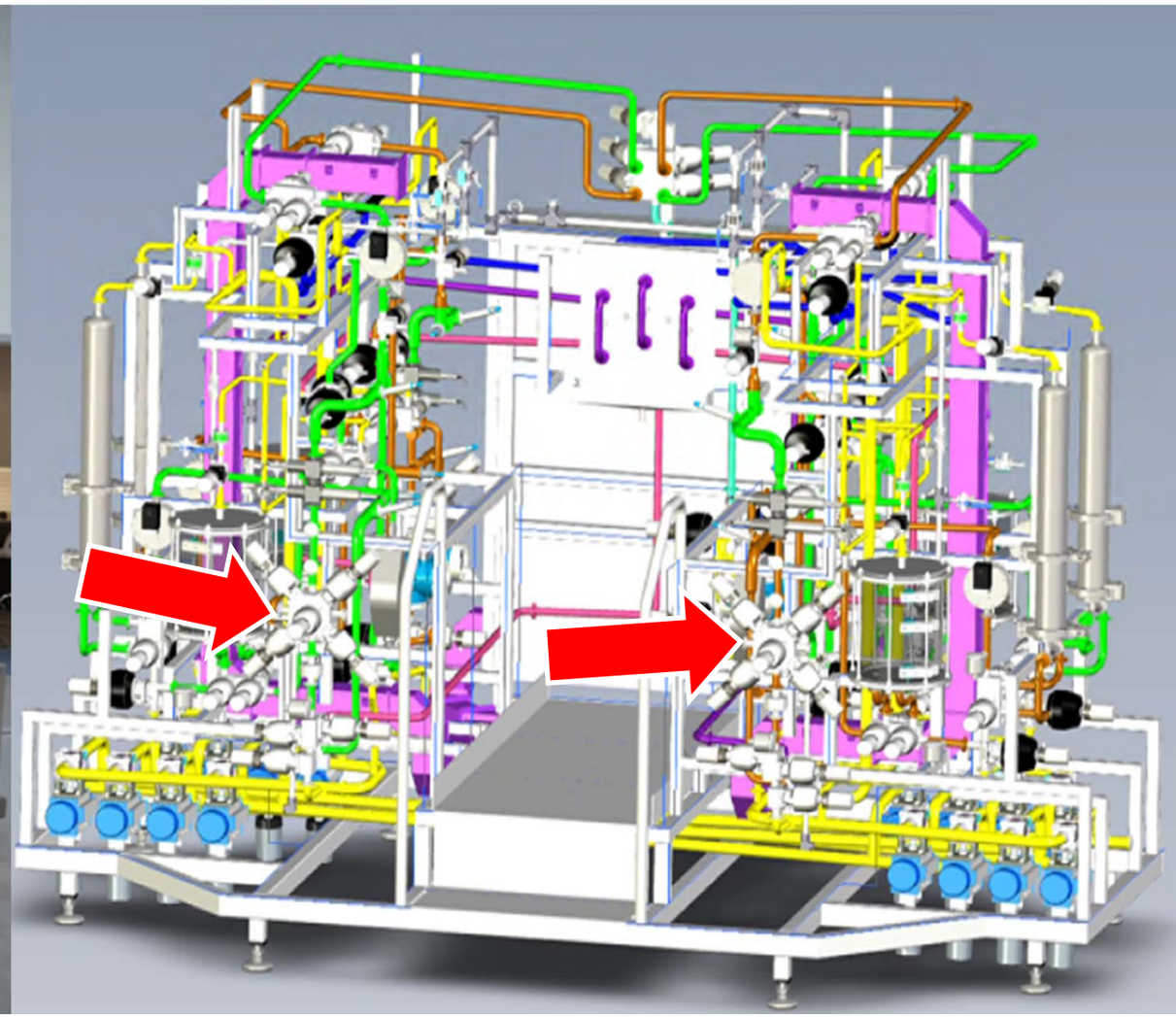

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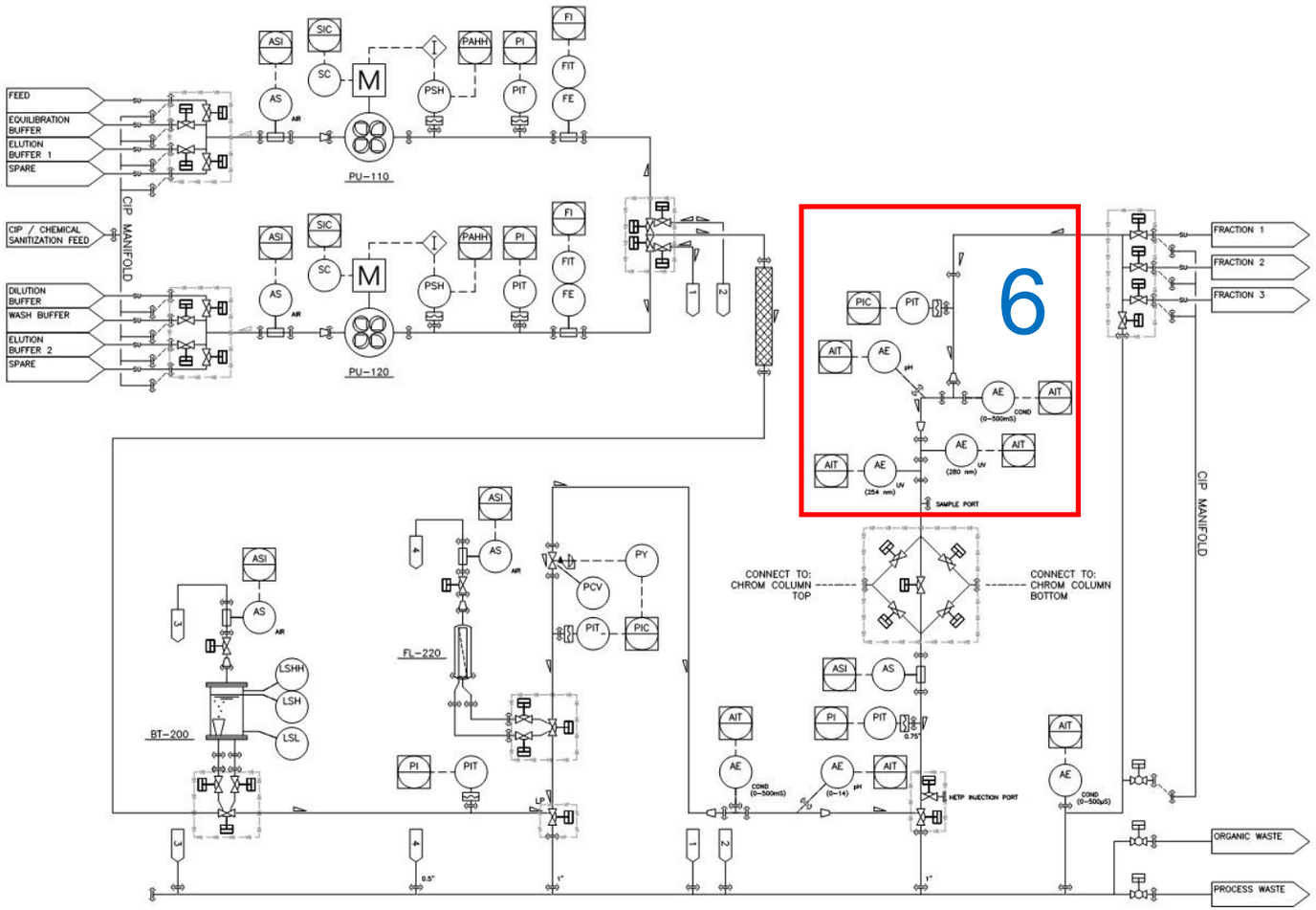

6. Post-Column Analytic Options

- Post-Column Conductivity
- Post-Column pH
- Post-Column UV Sensor (multiple wavelength instruments shown)
- Post-Column Pressure Transmitter

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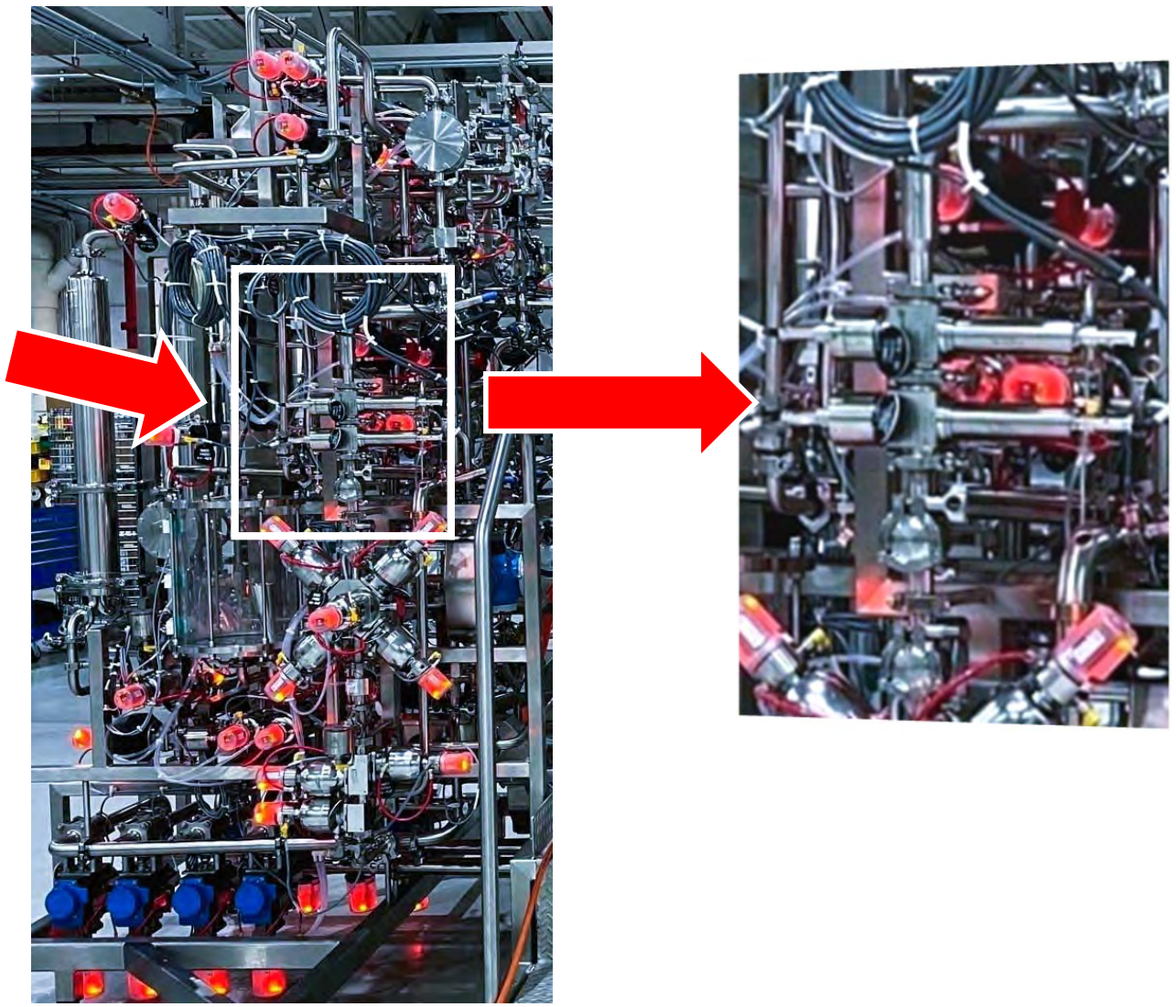

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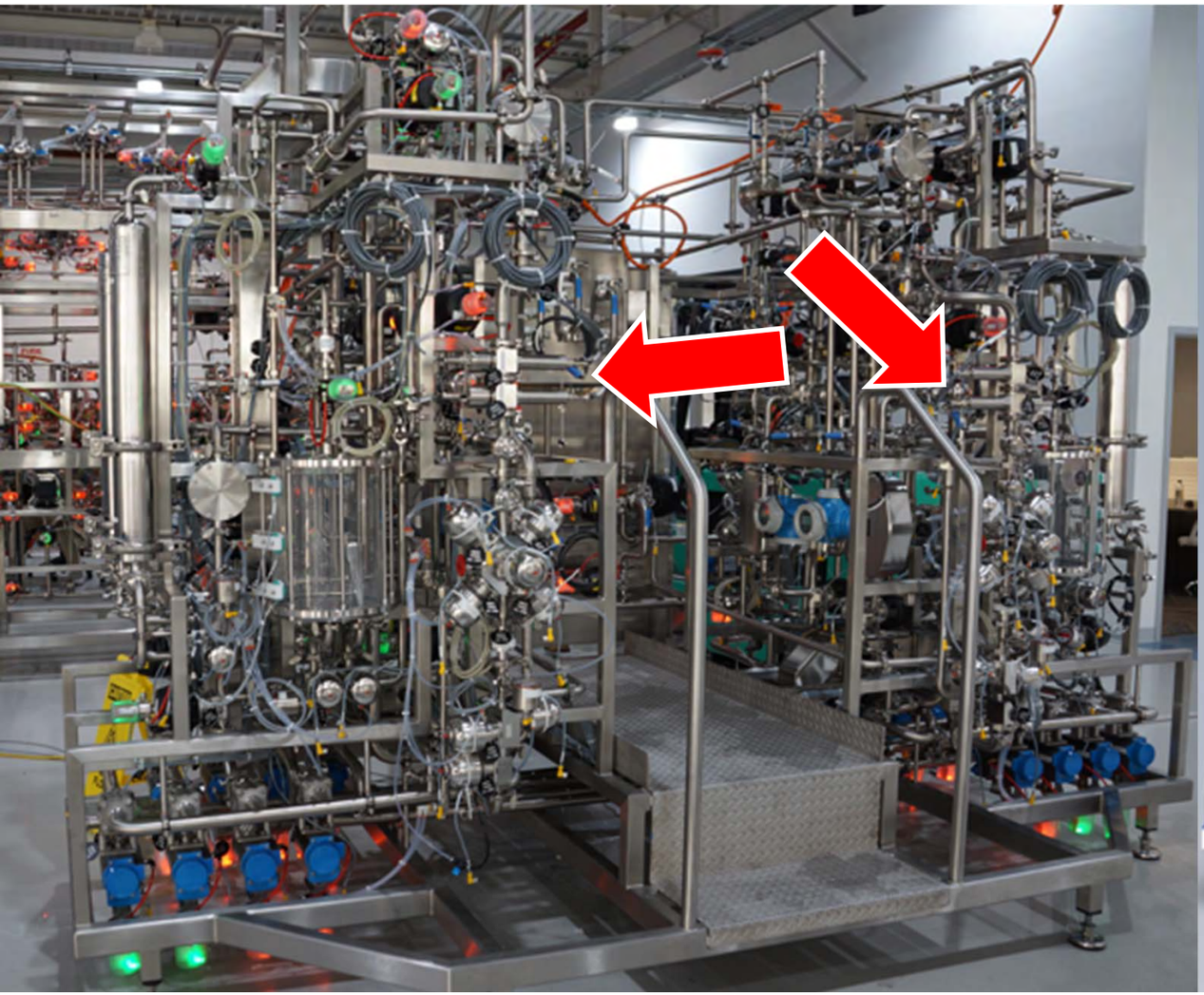

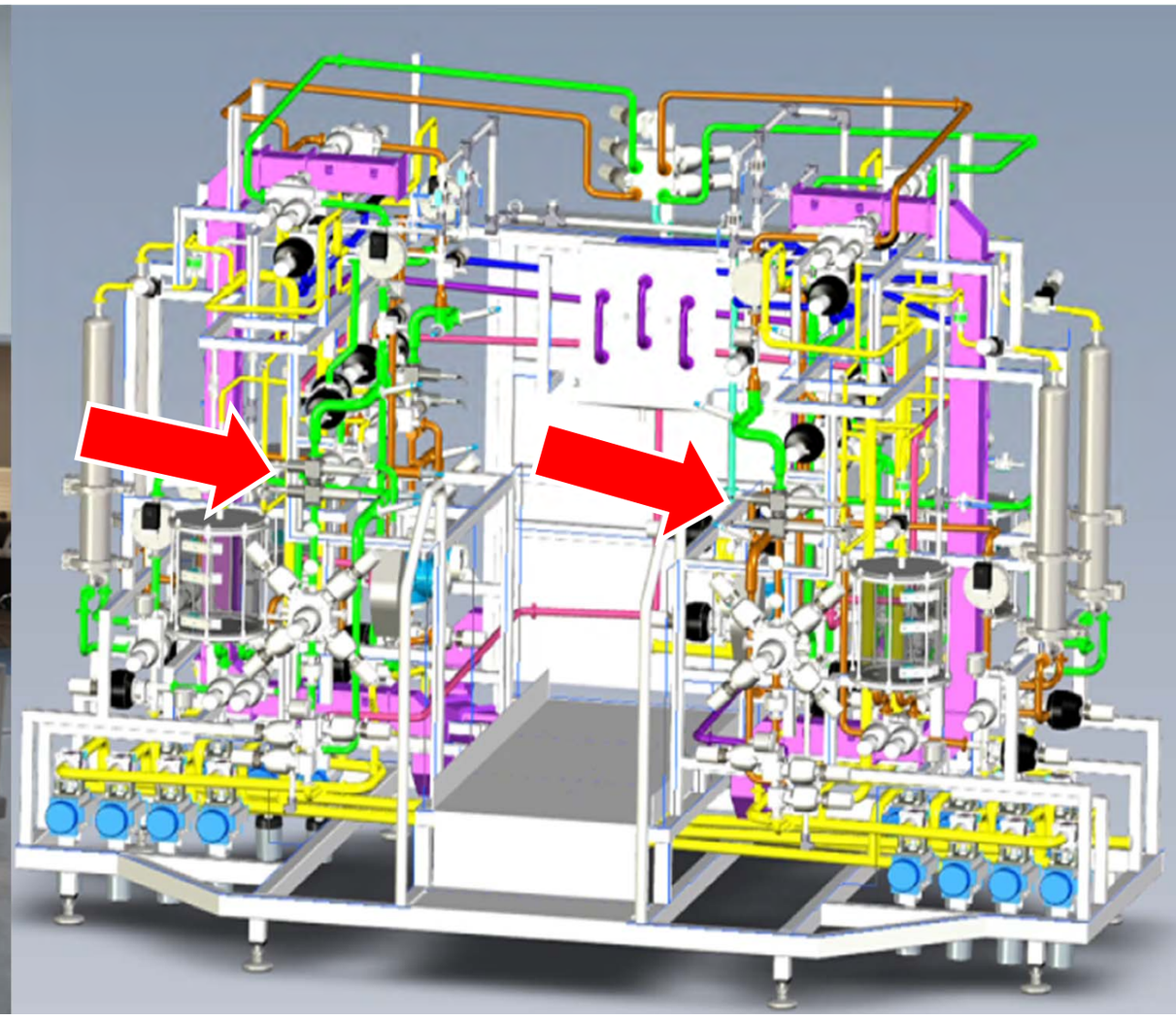

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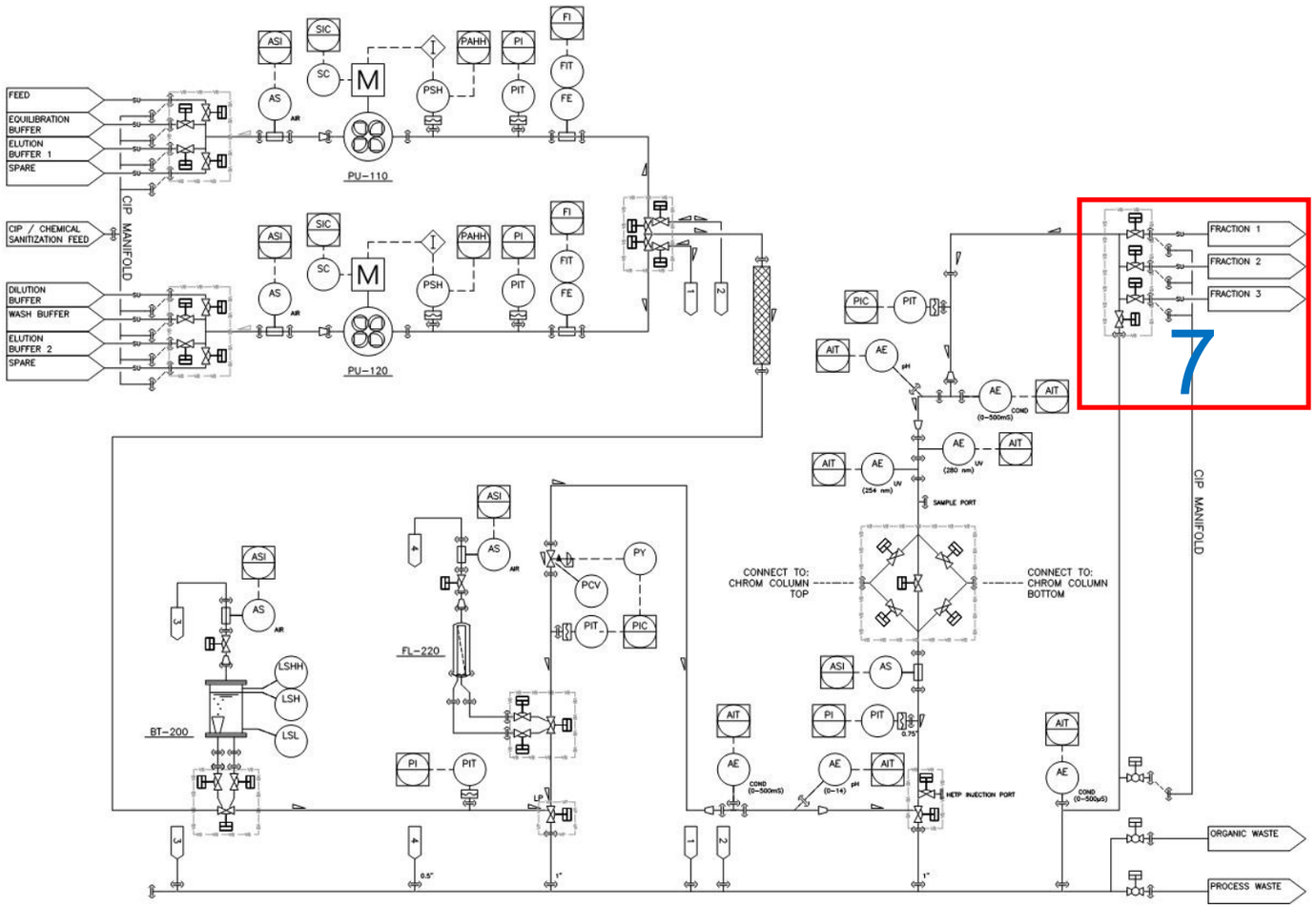

7. Fractionation Valves

- Customizable Fractionation Valve quantity
- Fractions can be programmed based on any post column analytical event or time.

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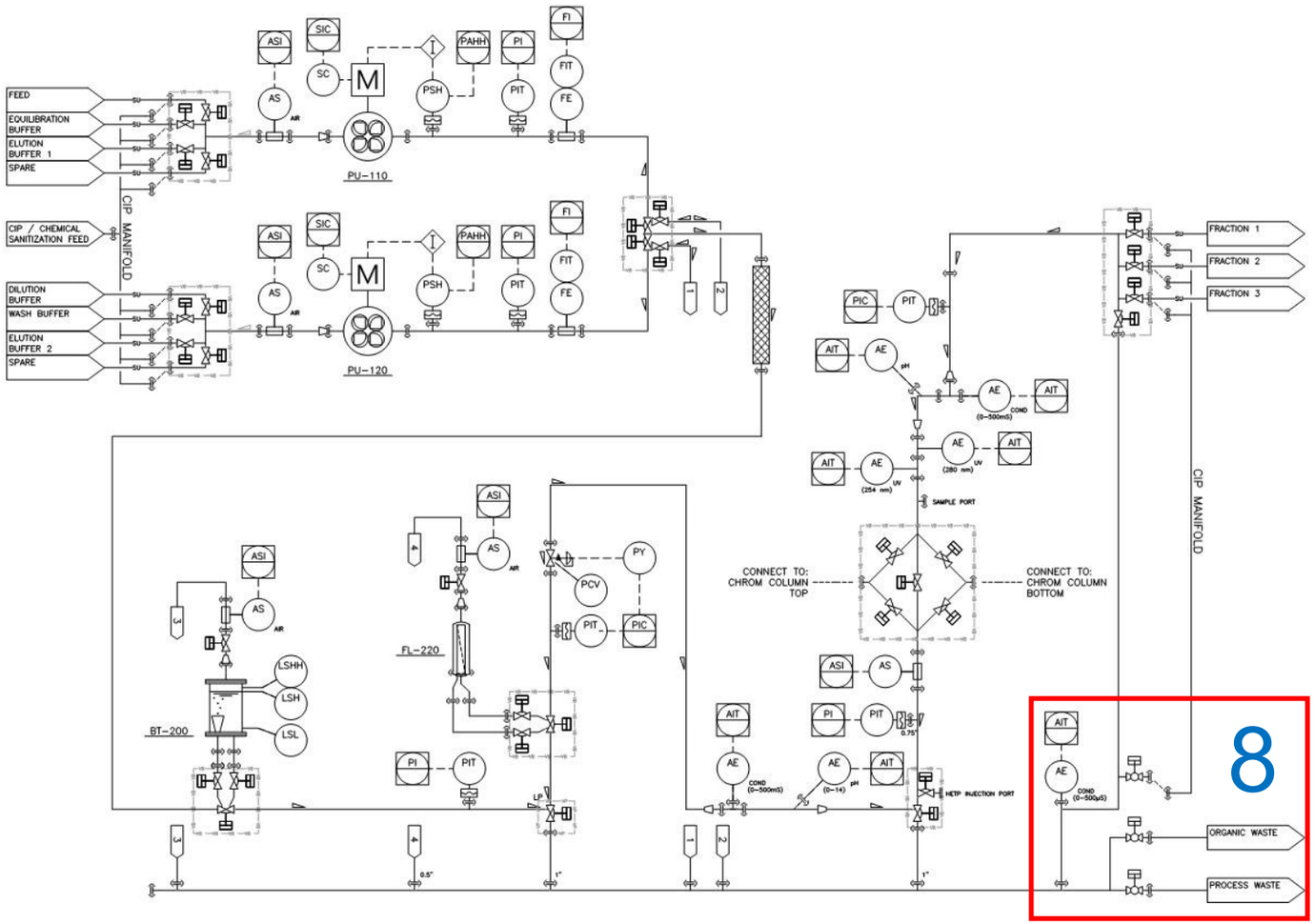
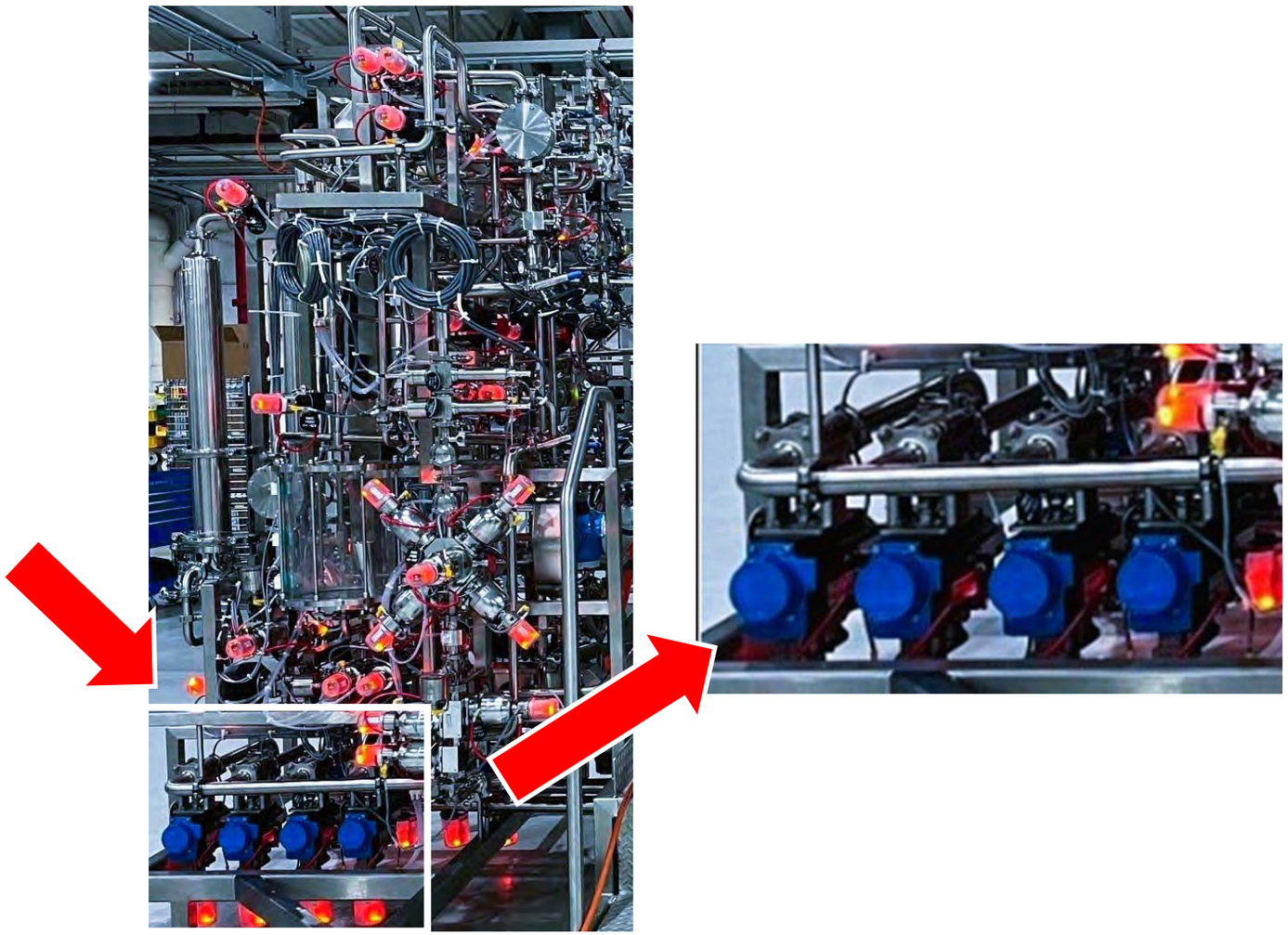


8. Rinse Conductivity & Waste Segregation Valves

- CIP rinse conductivity
- Waste can be segregated to specific drains based on phase step.
- Additional waste segregations can be added



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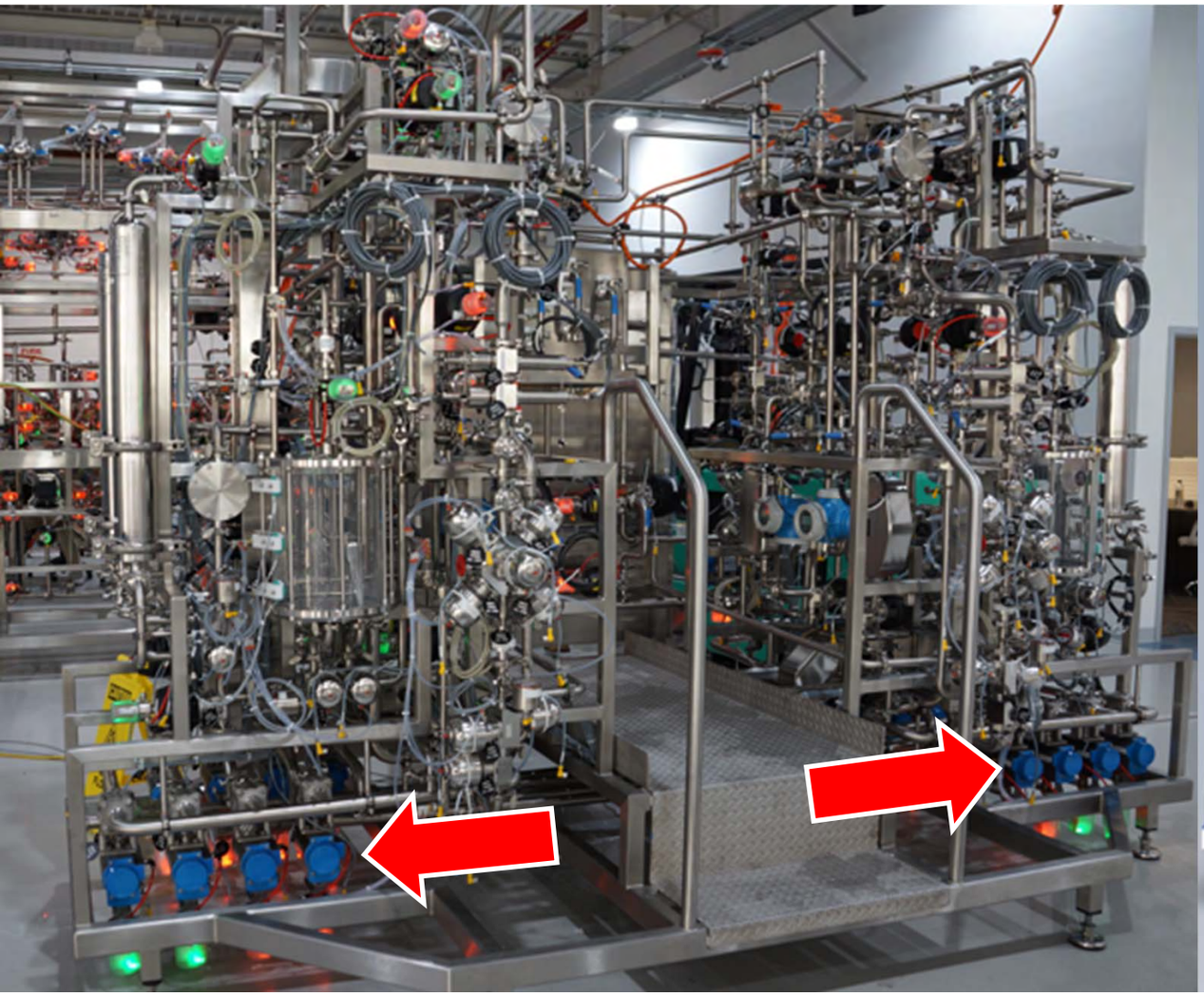

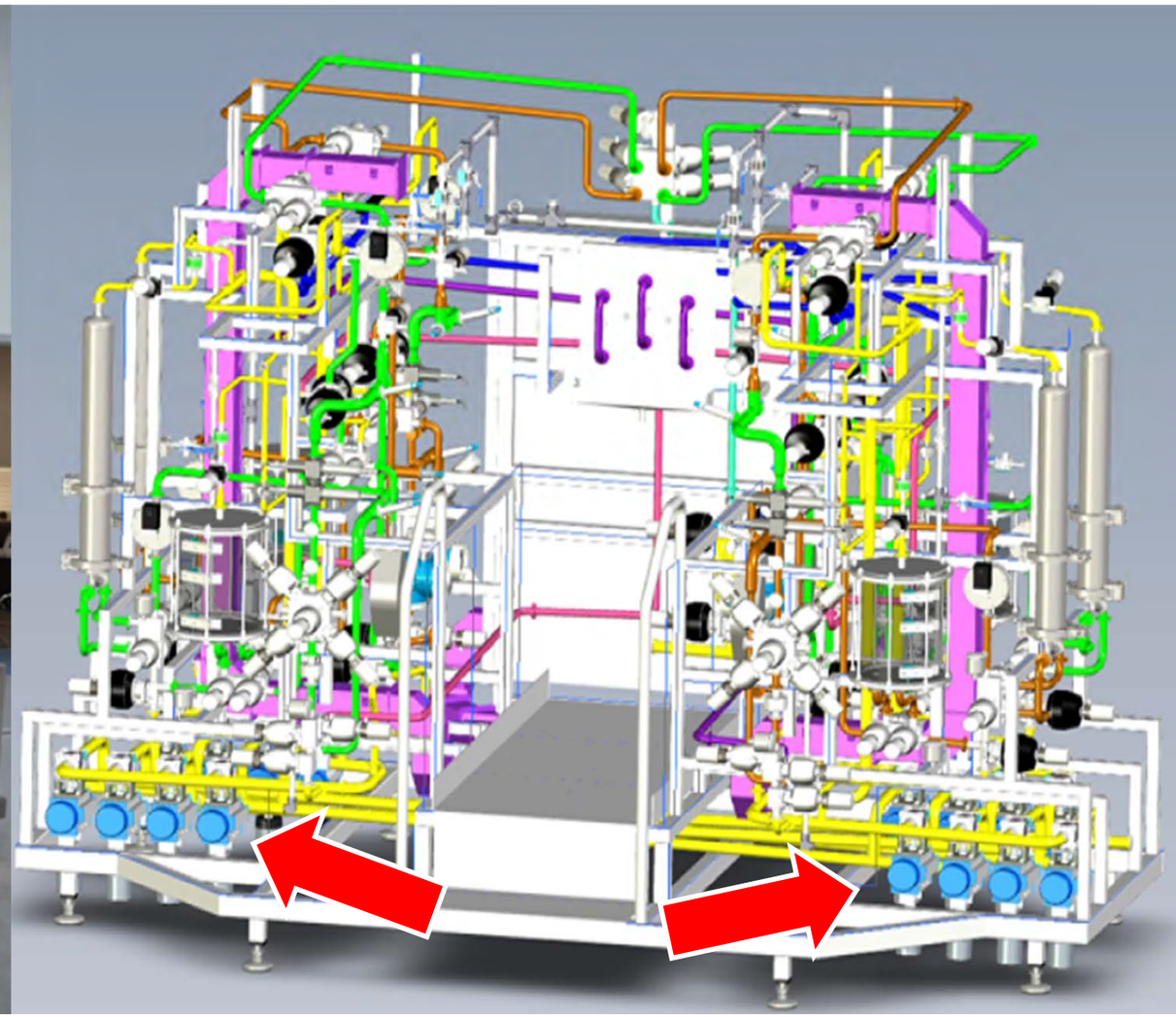

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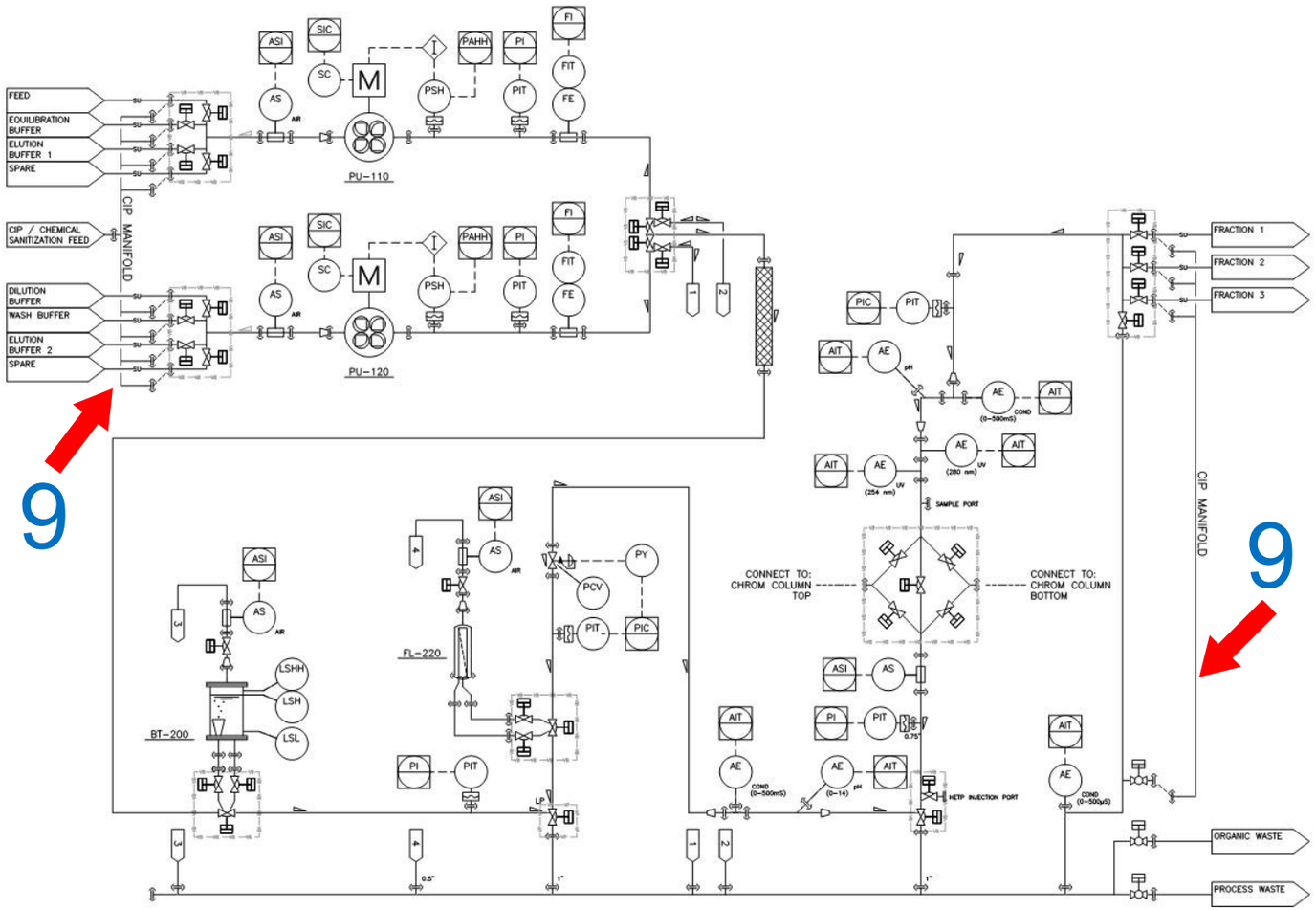

9. CIP / Sanitization Manifolds

- Ensure CIP, Sanitization & Rinsing through feed and fractionation valves
- Remain in place for liquid filled layup
- Typically made up of multiple sections for ease fit-up during installation

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Customizable Options

- Integration into hard-piped delivery and product storage
  - This can include steam sanitization of feed and product valves
- Modifying the number of feed and/or fractionation valves/connections
- Feed Heat Exchangers for heating or cooling
- Process Air or Nitrogen assisted purging
- HETP (column integrity) calculations integrated into control system
- Swapping the order of Bubble Trap & Filter
  - Benefit of order is a theoretical debate
- Addition of automatic or NovaSeptum type samplers downstream of column

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Where BPES fits in the market

Within the chromatography marketplace, BPES' focus is providing customers with **high quality, customized process skids** that support the functionality of their chromatography columns, by:

- Delivering product, equilibration, elution, washing and regeneration streams at precise flows and volumes
- Ensuring the quality of the delivered streams through reduction of entrained particles and air (including inline air sensing)
- Providing necessary chemical/physical property measurements within the chromatography column feed and product streams
- Applying strict design and fabrication criteria that facilitates proper cleaning, sanitization and storage of the process lines within the system.

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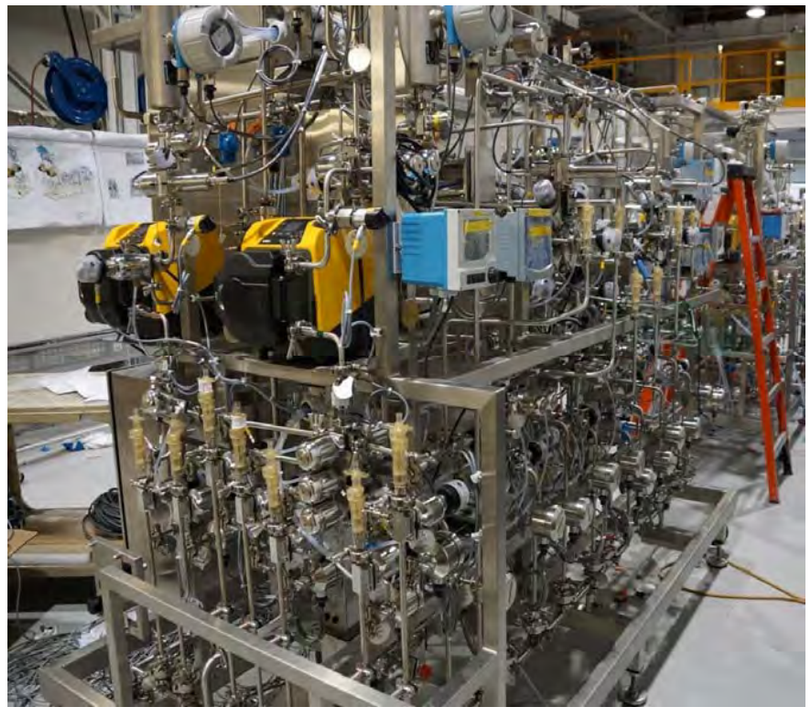
Where BPES fits in the market

- ☑ Low Pressure Liquid Chromatography (LPLC) Skids ← *< 300 psi*
- ☑ High Performance (Pressure) Liquid Chromatography (HPLC) Skids ← *> 300 psi*

- ☑ Batch Chromatography Skids
- ☑ Single Column Chromatography
- ☑ Multi-column Chromatography

- ☐ Chromatography Columns
- ☑ Chromatography Column Packing Skids



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