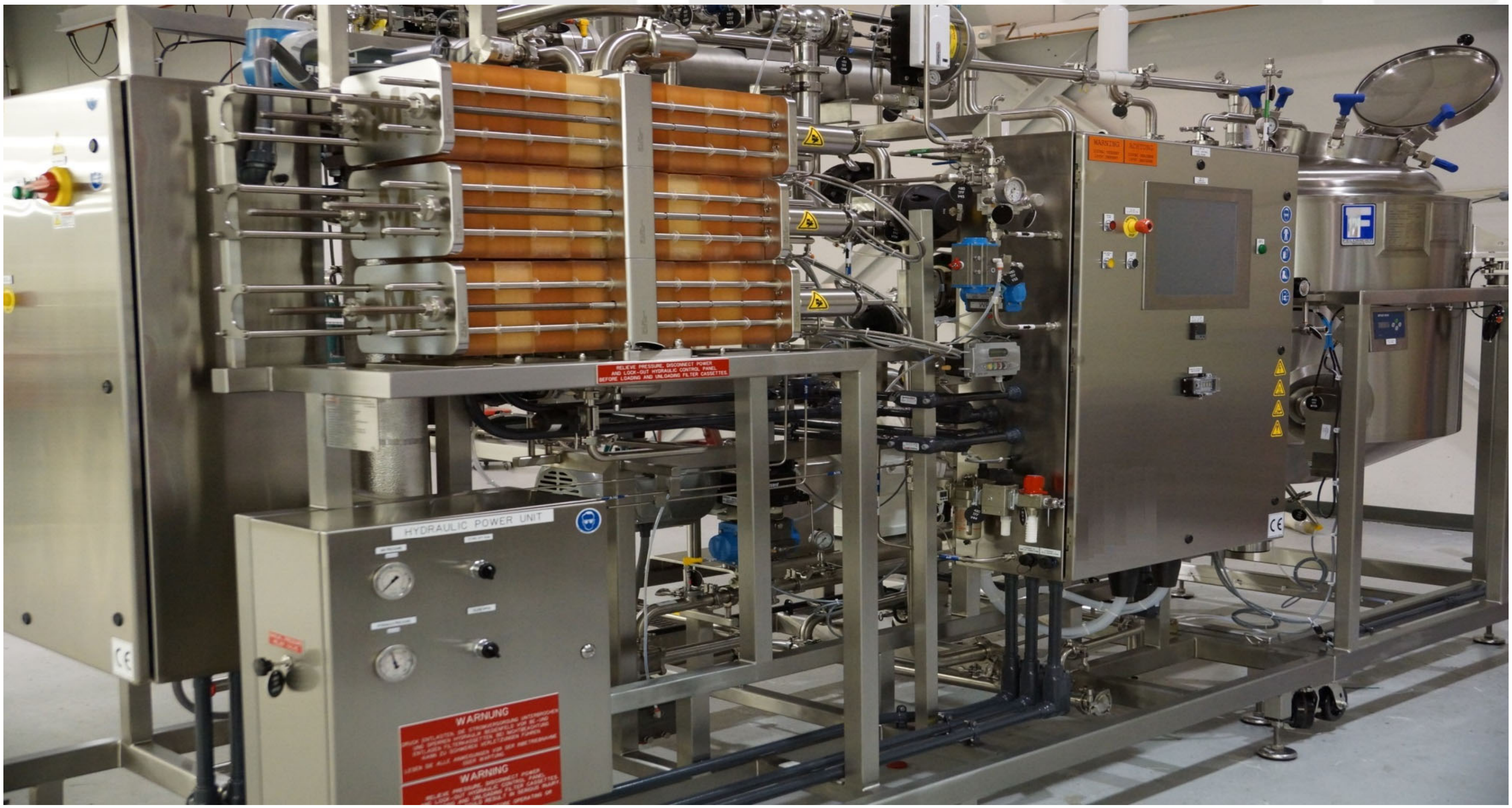
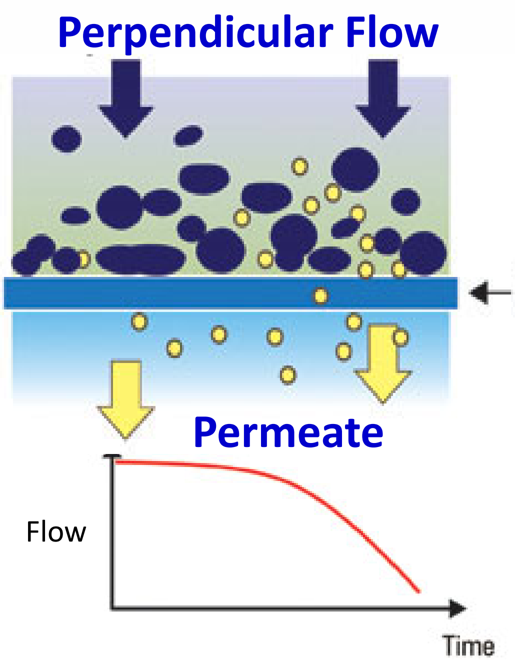
GENERAL OVERVIEW PRESENTATION : TANGENTIAL FLOW FILTRATION



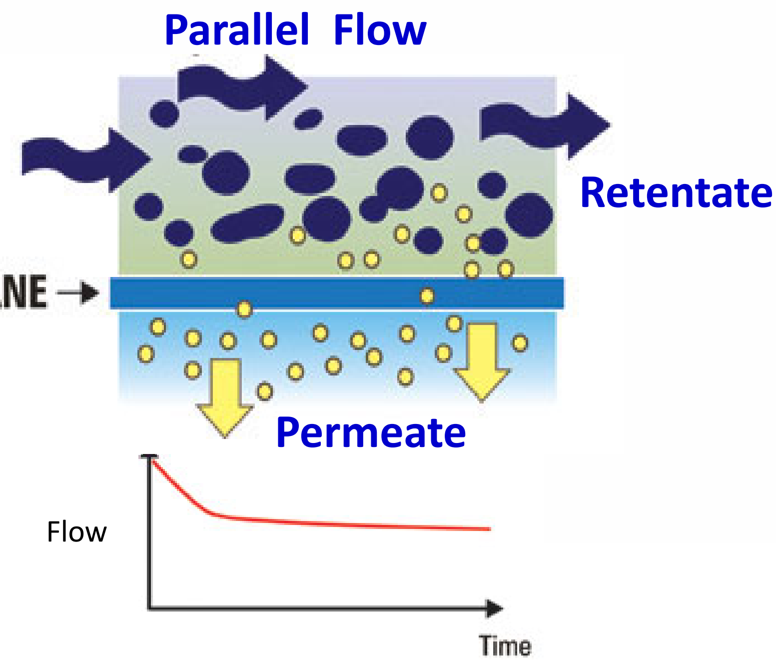
COMMON TYPES OF FILTRATION

Normal Flow Filtration (NFF)
- Cartridge or "Dead-ended" Filtration
- Flow is perpendicular to the filter media
- All fluid passes through the media
- Particles are retained in/on filter

Tangential Flow Filtration (TFF)
- Cross-flow Filtration
- Flow is tangential to the filter surface
- A small percentage of the fluid flows through the filter media
- Retained particles are swept away from filter surface

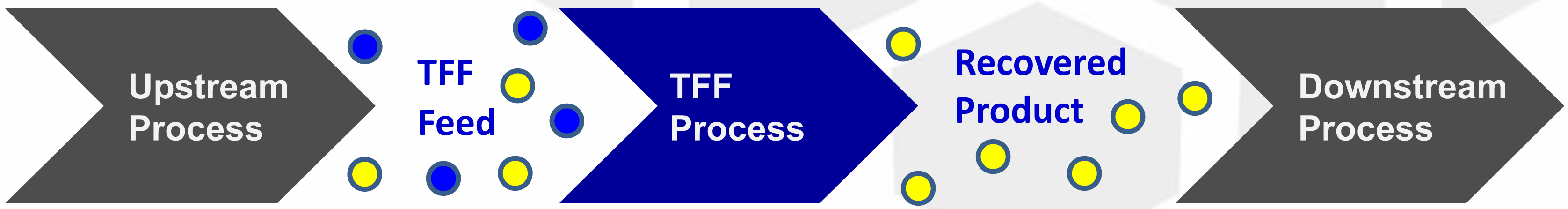


MEMBRANE



TFF PROCESS APPLICATIONS

Used as a step in a multi-step manufacturing process
Objective is to Process Feed Fluid from Initial Conditions to Final Specifications



Upstream

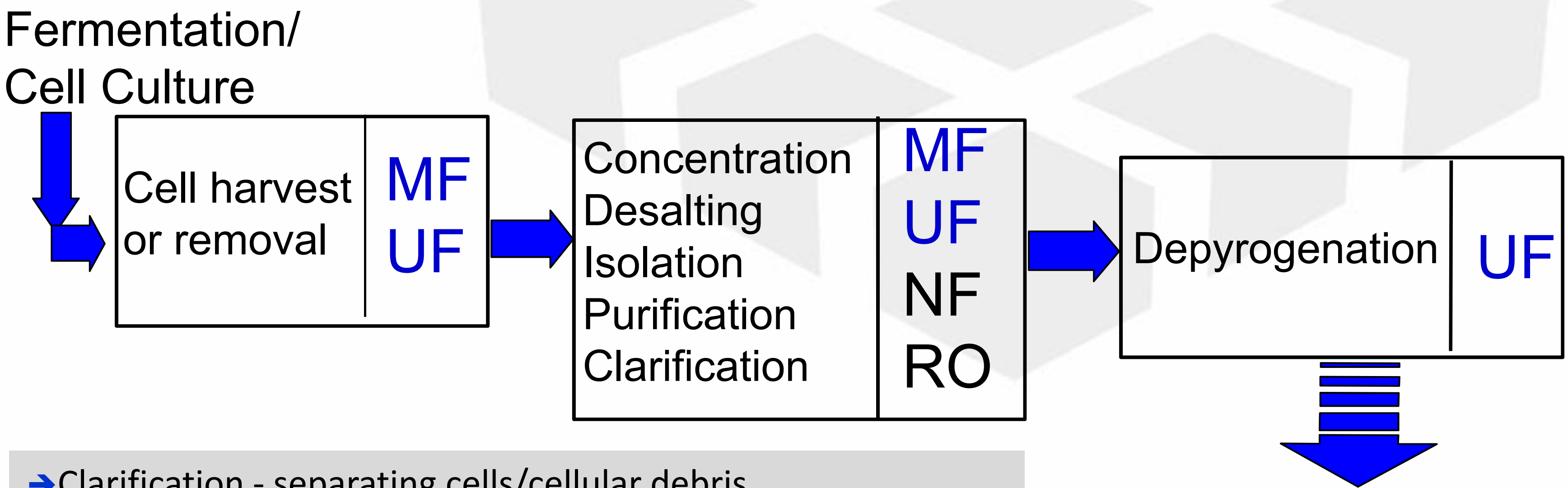
- Cell Harvesting (concentration) | Bacterial or yeast cells (product is intra-cellular)
- Cell Culture Clarification | Mammalian cell (product is extra-cellular)
- Cell Culture Perfusion | Mammalian cells

Downstream

- Protein Concentration
- Buffer Exchange (Diafiltration)

Clarification | Product Passes Through Membrane, Large Particles Retained
Concentration | Product Retained, Buffer Passes Through Membrane
Diafiltration or Buffer Exchange | Product Retained, Buffer Passes Through Membrane and New Buffer Added to Product

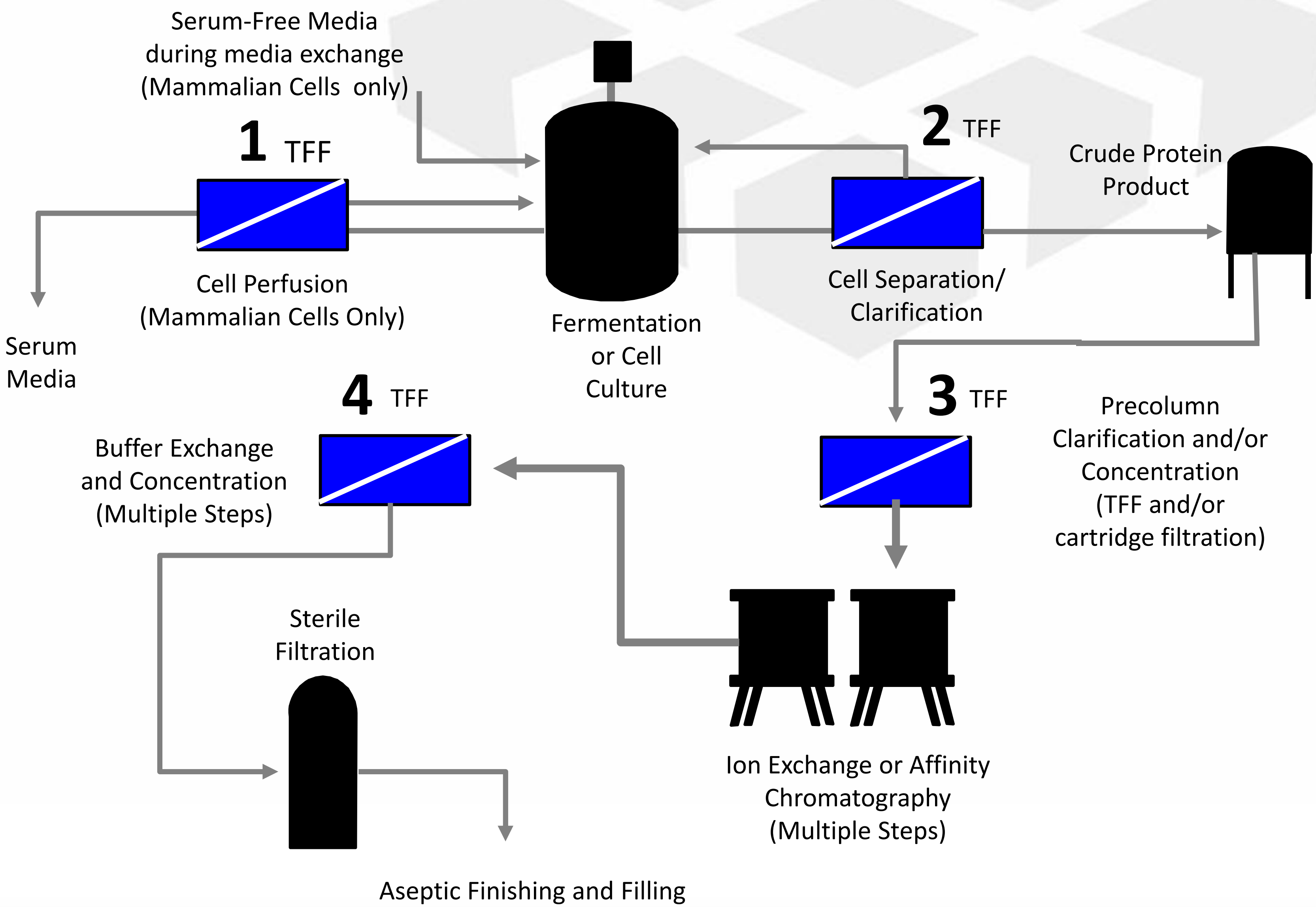
TFF APPLICATIONS IN BIOPHARM



- ➔Clarification - separating cells/cellular debris
  - CHO cell harvest and perfusion
  - CHO cell removal
- ➔Concentration - removing water/buffer
  - prior to/after chromatography
  - prior to fill and finish
- ➔Buffer Exchange/Desalting - removing salts
  - prior to/after chromatography
  - prior to fill and finish
- ➔Purification - removing contaminants
  - removal of native proteins

Antibiotics
Blood fractions
Peptides
Proteins
Vaccines
Radiopaques

PURIFYING EXTRACELLULAR PROTEINS



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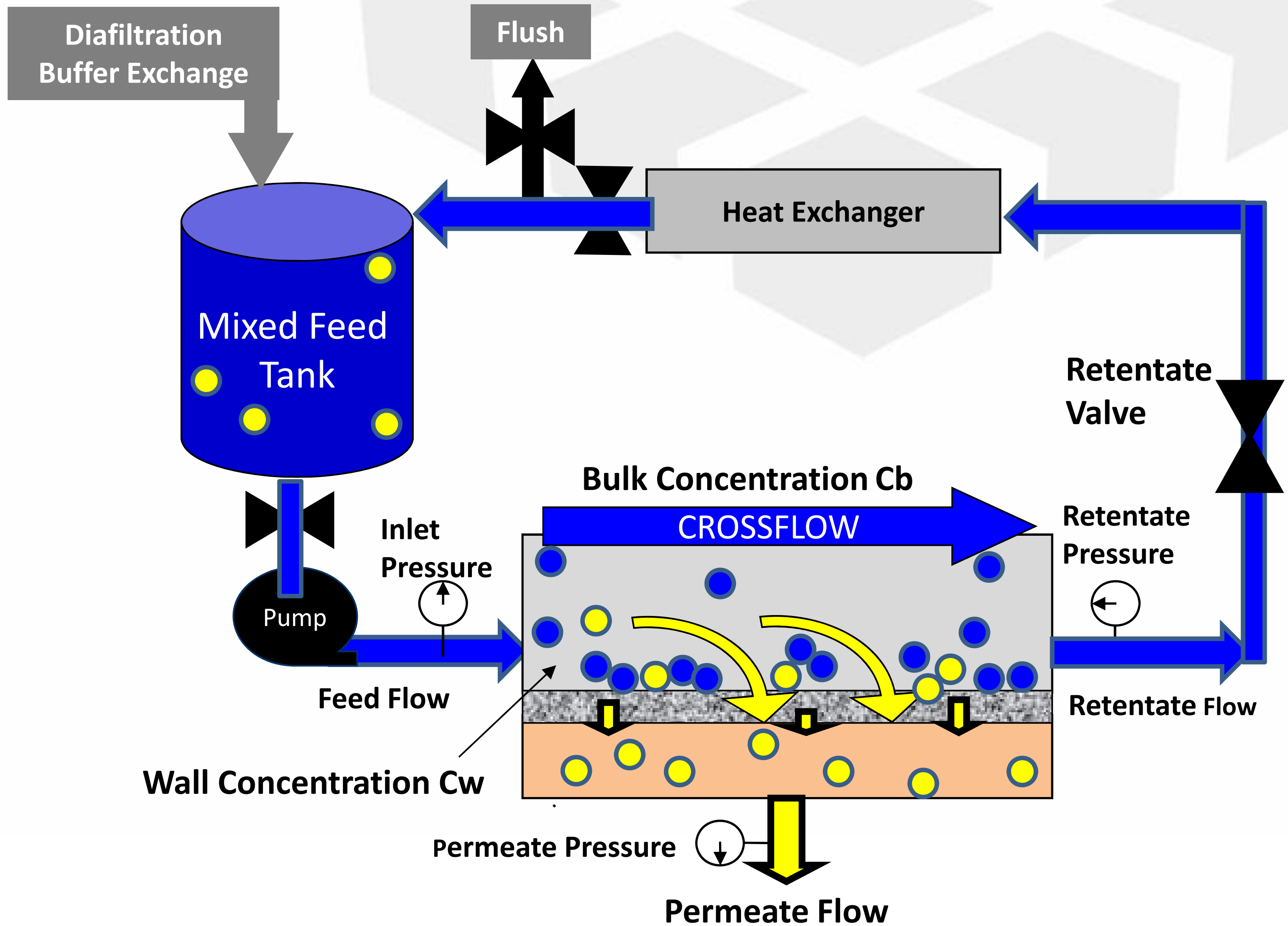
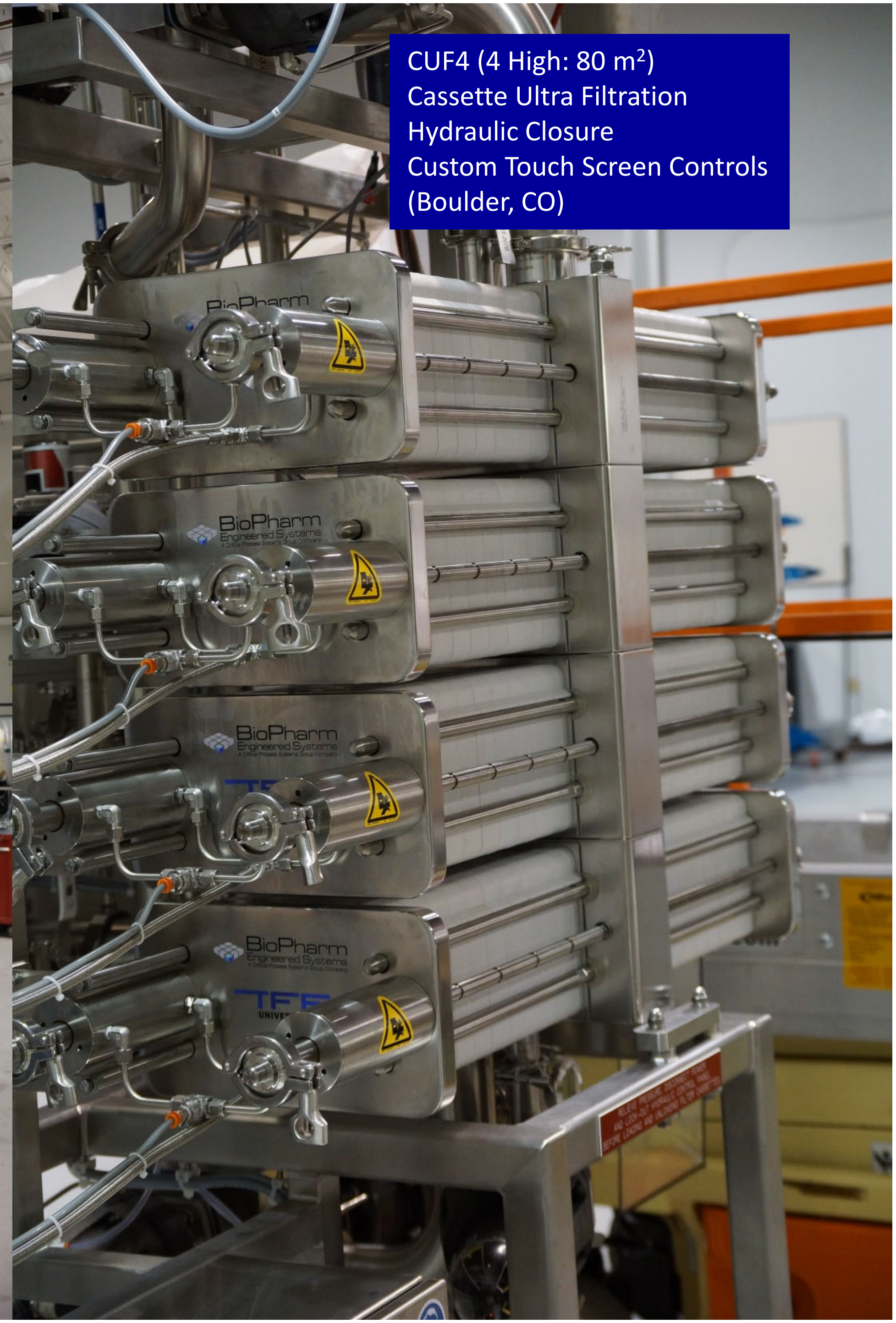
BASIC TFF SYSTEM LAYOUT





CUF4 (4 High: 80 m^2)
Cassette Ultra Filtration
Hydraulic Closure
Custom Touch Screen Controls
(Boulder, CO)

TFF SYSTEM DESIGN CONSIDERATIONS

Define the purpose of the TFF process:

- Separation of biomolecule product can occur by choosing a membrane that retains the product while passing any lower molecular weight contaminants.
- Alternately, a membrane can be chosen that passes the product while retaining the higher molecular weight components.

Choose the membrane MWCO:

- The Molecular weight cutoff (MWCO) of a membrane is defined by its ability to retain a given percent of a molecule in solution (typically 90% retention).
- To retain a product, select a membrane with an MWCO that is 3-6 times lower than the molecular weight of the target protein.
- For fractionation, select a membrane that is lower than the molecular weight of the molecule to be retained but higher than the molecular weight of the molecule you are trying to pass.

TFF SYSTEM DESIGN CONSIDERATIONS

Determine the required membrane area for the application:

- Choosing an appropriate TFF system depends on the total inlet volume, required processing time, and desired final outlet volume.
- Alternately, a membrane can be chosen that passes the product while retaining the higher molecular weight components.

The following equation is used to calculate the required membrane area for the process:

A = V / (J * T)

Where:

A = Membrane Area (m2)
V = Volume of filtrate generated (l)
J = Filtrate flux rate [liters/m2/hr (LMH)]
T = Process Time (hrs).

Example: What TFF system should one use to concentrate 2000 L to 200L in 2.5 hrs? Assume average filtrate flux rate of 50 liters/m2/hr (LMH).

V = 2000 L – 200L = 1800L

$$A = \frac{1800\ \text{L}}{50\ \text{LMHx}\ 2.5\ \text{hr}} = \frac{1800}{125} = 14.4\text{m2}$$

TFF SYSTEM OPTIMIZATION

System Optimization:

- The maximum efficiency of a TFF separation depends upon the optimum combination of feed flowrate (Q_{feed}) and the pressure across the membrane surface, also called the Transmembrane Pressure (TMP).

Transmembrane Pressure (TMP):

- TMP is the driving force for liquid transport through the TFF membrane, calculated as the average pressure applied to the membrane minus any filtrate pressure. (In most cases the pressure at the filtrate port equals zero).

The following equation is used to calculate the Transmembrane Pressure (TMP) and Water Permability:

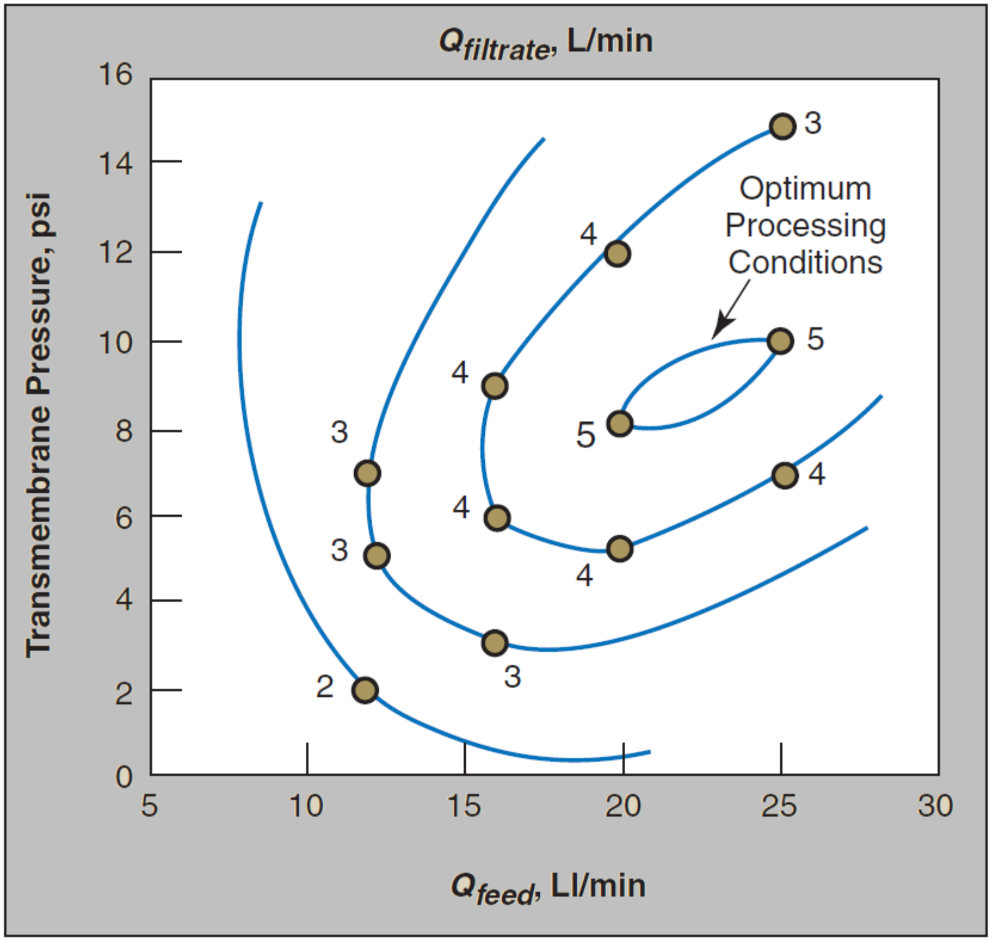
$$TMP = \frac{(P_{feed} + P_{retentate})}{2} - P_{Permeate}$$

$$\text{Water Permeability} = \frac{\text{Permeate Flux Rate (LMH)}}{TMP}$$

Feed Flowrate (Q_{feed}) Considerations:

- For non-shear sensitive feed products the optimum value for Q_{feed} for TFF is normally driven by the maximum inlet pressure of the TFF Membrane. For shear sensitive feed products (eg: mammalian cells) the optimum value for Q_{feed} is the maximum flow below the shear force at which cells can rupture.

TFF SYSTEM PROCESS MAP



[redacted]

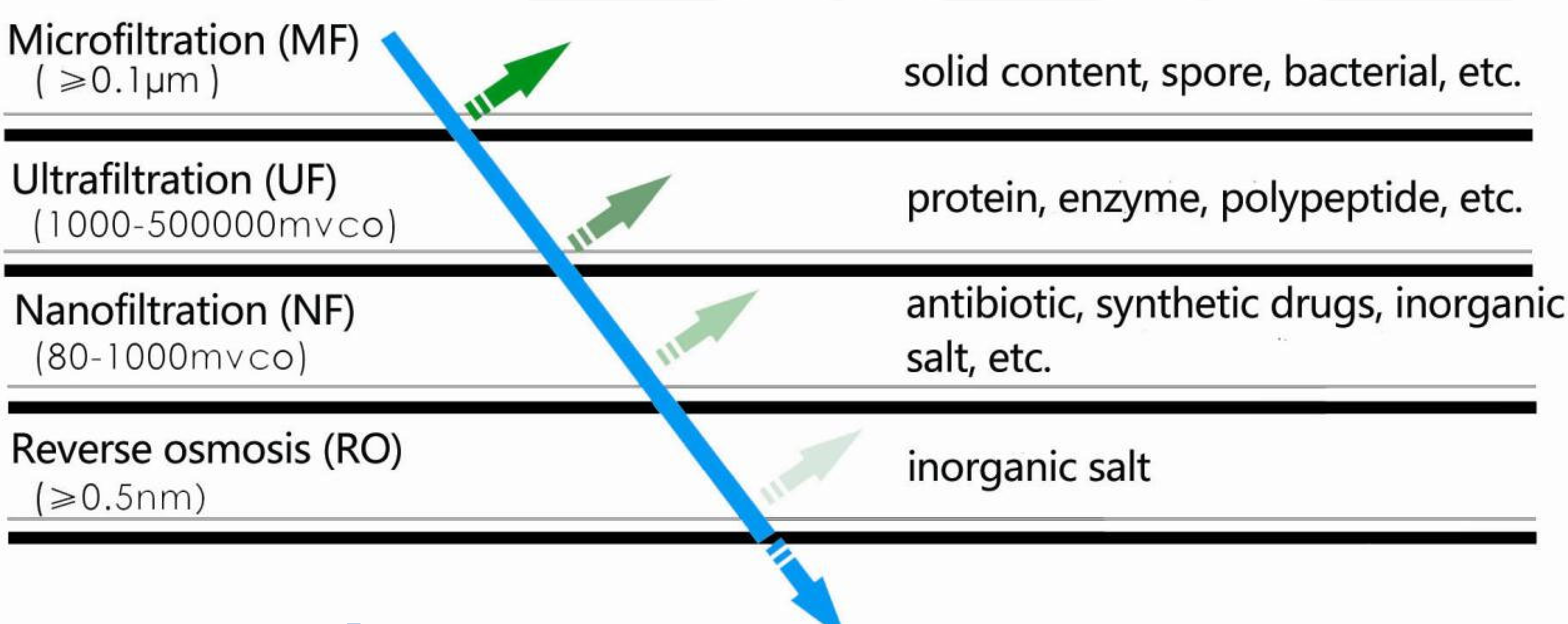
$Q_{filtrate}$, L/min	Q_{feed}, L/min	TMP, psi
2	12	2
3	12	5
3	12	7
3	16	3
4	16	6
4	16	9
4	20	5
5	20	8
4	20	12
4	25	7
5	25	10
3	25	15

Nomenclature

P_{fi} = feed pressure on inlet side of membrane, psig
P_{fo} = feed pressure on outlet side of membrane, psig
P_{po} = permeate (or filtrate) pressure on outlet side of membrane, psig
Q_{feed} = feed volumetric flowrate, L/min
$Q_{filtrate}$ = filtrate volumetric flowrate, L/min
TMP = transmembrane pressure, psi (Eq.1)

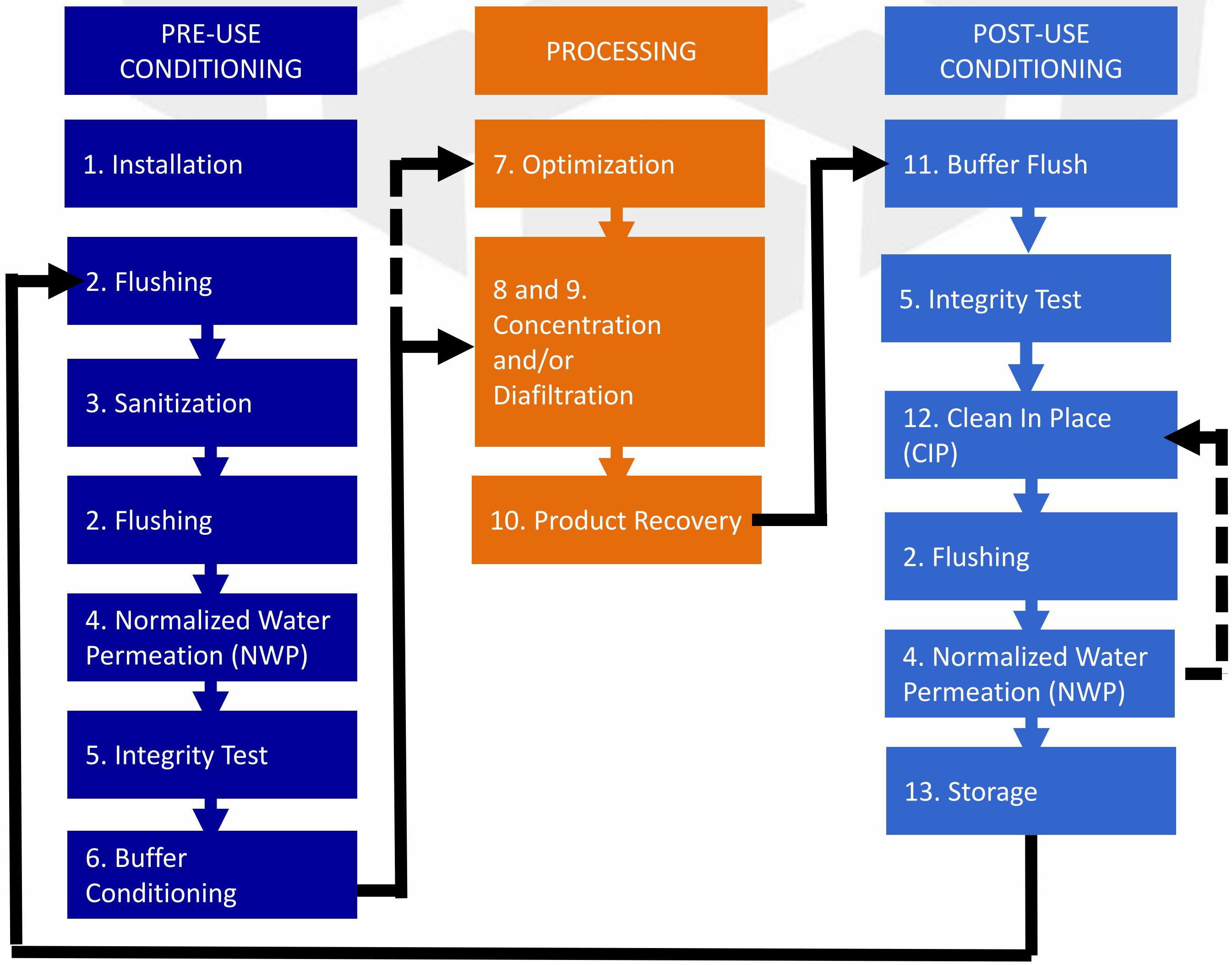
[1] Kent Iverson, Iverson Consulting w/Solution Pharmaceutical Advisors, "Troubleshooting Tangential Flow Filtration", CEP Magazine, June 2003.

MEMBRANE TYPE BY CUTOFF



MF	Microfiltration	for substances whose cutoff diameter is more than 0.1 um. Pre-filter of UF/NF/RO
UF	Ultrafiltration	for soluble substances whose molecular weight cutoff ranges between 1K and 500K.
NF	Nanofiltration	for soluble substances whose molecular weight cutoff 80 – 1K and diameter ~1 nm.
RO	Reverse Osmosis	for water and other small molecules whose cutoff diameter is more than 0.5 nm.

THE TFF PROCESS SEQUENCE



MAXIMIZING PRODUCT RECOVERY

Not all the fluid in the system is recoverable by draining or blow-down of the system

Minimizing Unrecoverable Volume:

- Varies with filter and installed area
- May be as much as 10-20% of minimum working volume
- Without a buffer flush, could lead to 10-20% loss if product is concentrated to the minimum working volume, depending upon system design

Maximizing Yield by Over-Concentrating

- If possible, "over-concentrate" the product past the desired final concentration.
- Then drain the system thoroughly--but some product stays behind.
- Then flush with a minimum working volume of buffer to dissolve product still left in the system.
- Drain out this buffer and add back to the concentrate, diluting the product solution to desired final concentration.

MAXIMIZING PRODUCT RECOVERY

EXAMPLE : YIELD WITH NO FLUSH

- System minimum working volume: 150 ml
- Product: 100 mg in 10 L starting volume
- Concentrated to 300 ml final volume
- Unrecoverable volume after draining: 25 ml
- Loss: 100 mg x (25 ml/300 ml) = 8.3 mg

Yield: 92%

EXAMPLE: YIELD WITH FLUSH AND CONCENTRATION

- System minimum working volume: 150 ml
- Product: 100 mg in 10 L starting volume
- Concentrated to 150 ml
- Unrecoverable volume after draining: 25 ml
- Loss: 100 mg x (25 ml/150 ml) = 17 mg
- Flush with buffer: 150 ml
- Add back: final volume still 300 ml
- New loss: 17 mg x (25 ml/150 ml) = 2.8 mg

Yield: 97%
Improvement: 5%

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TFF DESIGN CONSIDERATIONS

Things to think about when developing system specifications & P&ID's for TFF Systems:

Valves

Reduce the number of valves between the system tank and suction side of the pump.
Make sure there is a fail-safe position to relieve pressure in the event of an emergency shutdown or fault Fail open vs fail closed.
Reduce the number of valve position limit switches.
Consider block valves vs ported valves cost, delivery & footprint.

Pumps

Size for rated flow and pressure at 80% of pump capacity and less than 450 RPM
Make sure you have adequate upside for CIP if required.
Keep line size reductions to a minimum on the suction side of pump.
Gear motors will help keep motor speed up when running at reduced pump speed for end of batch processing

Continued Next Page

TFF DESIGN CONSIDERATIONS

Tanks

Conical bottoms vs tulip style for reduced volume processing
Retentate side wall "T-style" diptube return in place of tank top wall or J-tube style
Guided wave radar vs load cell and flex hose for tank level measurement
Bottom mount mag mixers in place of top mount agitation

Instrumentation

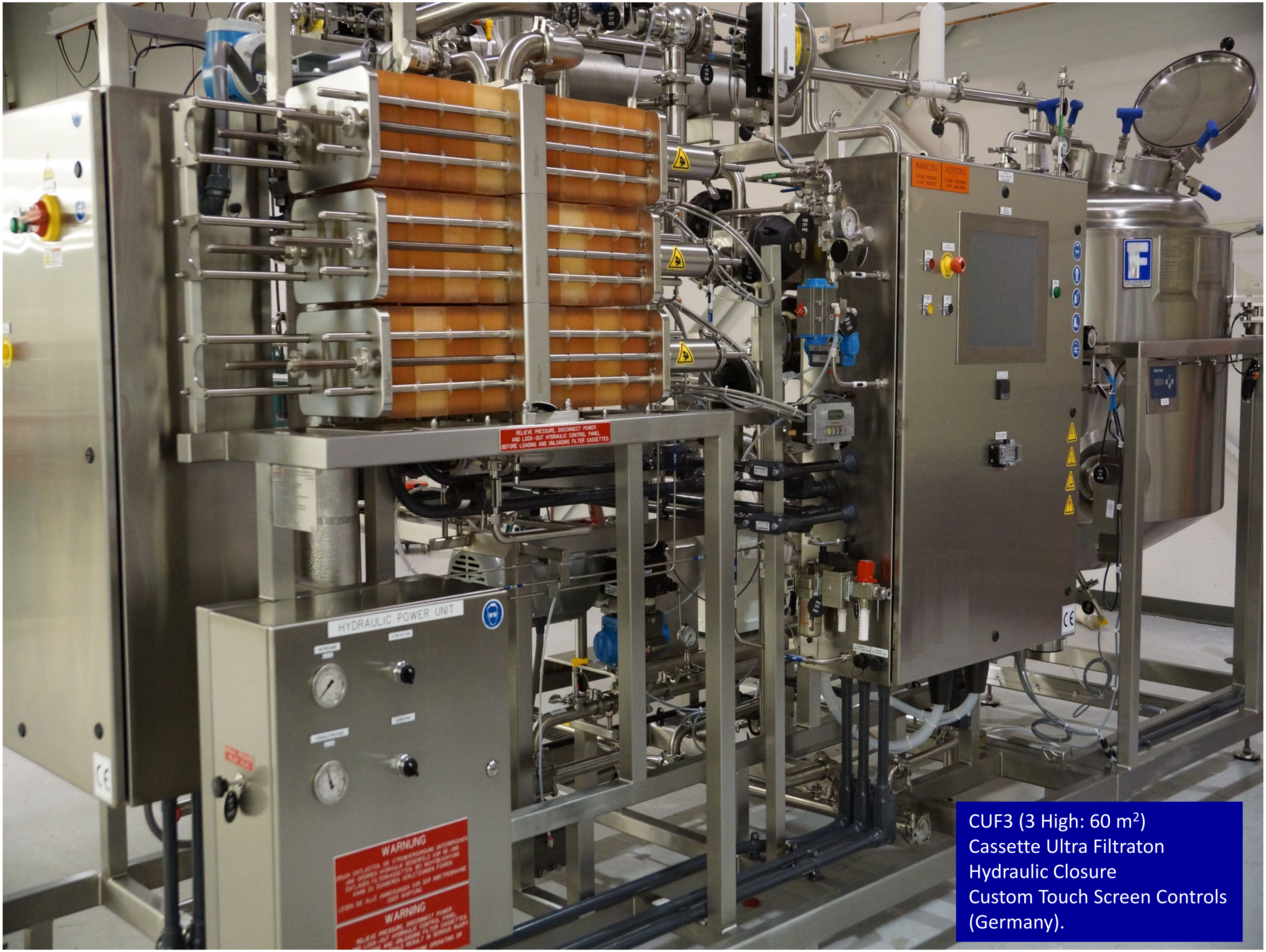
Size instruments so that operating range is within 50% of calibration span

Design

System Footprint and Installation Constraints
Delivery Considerations/Restrictions (Truck, Air, Ocean)
Minimum Hold-Up
Drainabilty (¼"/ft slopped lines.)

System Control

Manually Controlled System (Manual Valves, Drives, Etc.)?
Auto Controlled System (Alarms, Interlocks, Recipe's)?



CUF3 (3 High: 60 m^2)
Cassette Ultra Filtraton
Hydraulic Closure
Custom Touch Screen Controls
(Germany).