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science | evolved

Multiphysics Simulation of Isoelectric Point Separation of Proteins Using Non-Gel Microfluidics System

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Mission

To nurture and harvest scientific creativity
to produce life changing technologies

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Discoveries

- Energy Storage through Electrochemistry
- Chemical/Biological Defense and Countermeasures
- Environmental Remediation
- Medical Technologies

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Current Protein Diagnostic Techniques

Protein Analysis

-Enzyme-linked Immunosorbent Assays (ELISAs)

-2D Gel Electrophoresis

Protein Detection

-Mass Spectrometer

Expensive

Immobile

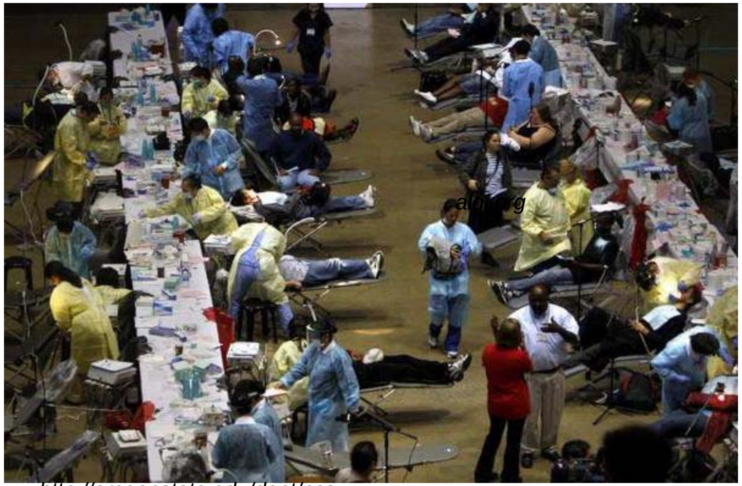
Hard to Maintain

Slow

Remote Area Diagnostic

http://www.defenseindustrydaily.com

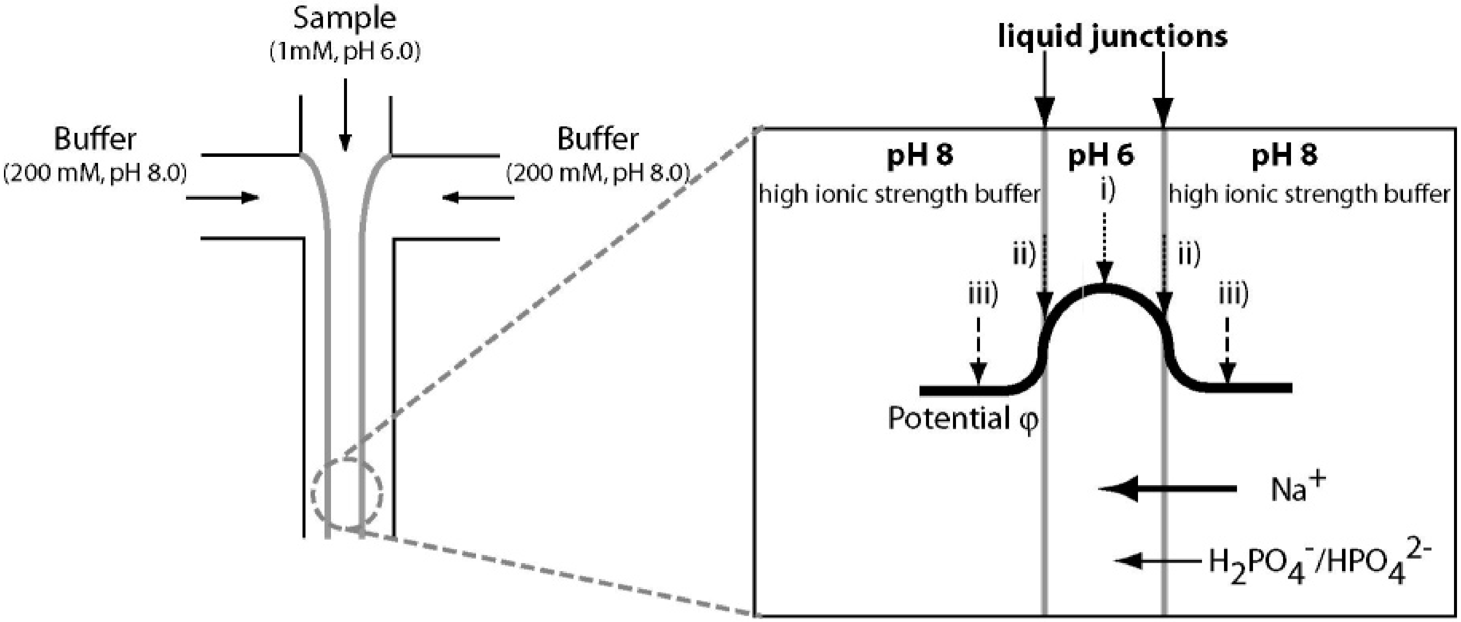
Yon M. Achieves. http://www.michaelyon-online.com



http://oregonstate.edu/dept/ncs

Isoelectric Point based Protein Separation

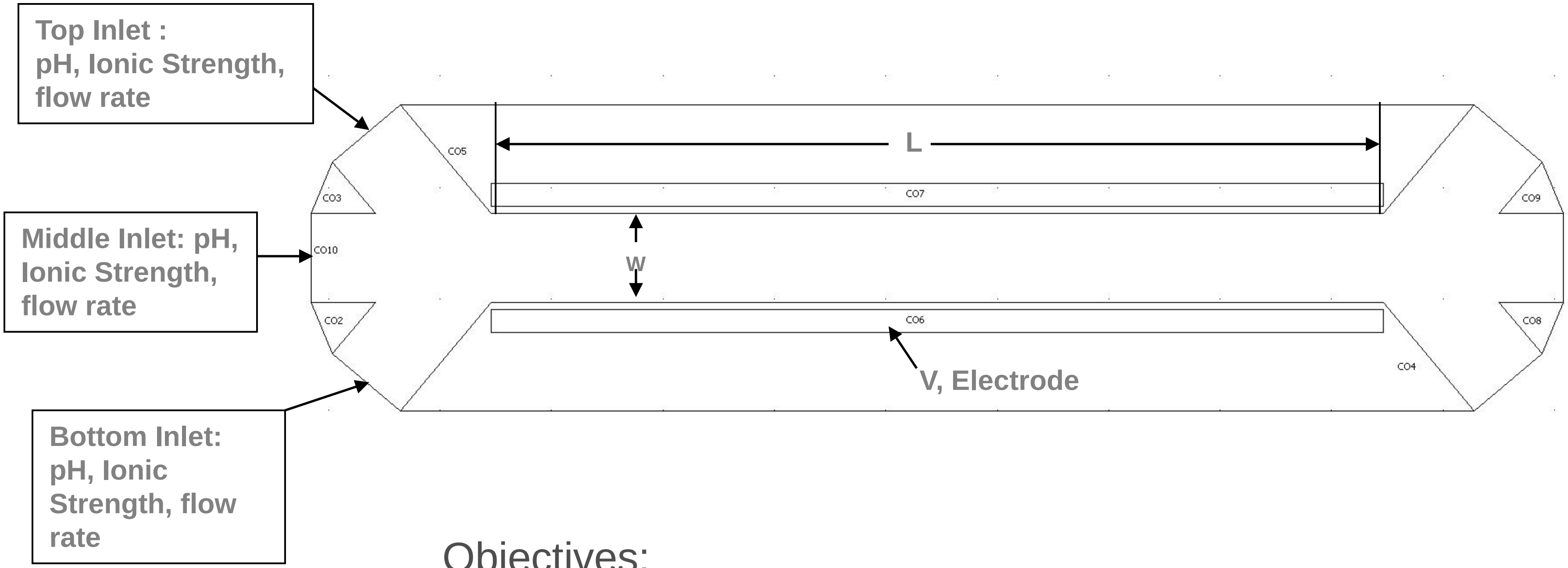
- Song et al. from MIT’s Biological Engineering Laboratory designed PI based protein separation



i) molecules with pI < pH 6.0 (always negative)
ii) molecules with pH 6.0 < pI < pH 8.0 (target)
iii) molecules with pI > pH 8.0 (always positive)

Song, Y., Hsu S., Stevens A.L., and Han J. Continuous-Flow pI-Based Sorting of Proteins and Peptides in Microfluidic Chip Using Diffusion Potential. Anal. Chem. (2006) 78(11): 3528-3536

Lynntech's Concept: Isoelectric Point based Protein Separation Chip



Top Inlet :
pH, Ionic Strength, flow rate

Middle Inlet: pH, Ionic Strength, flow rate

Bottom Inlet: pH, Ionic Strength, flow rate

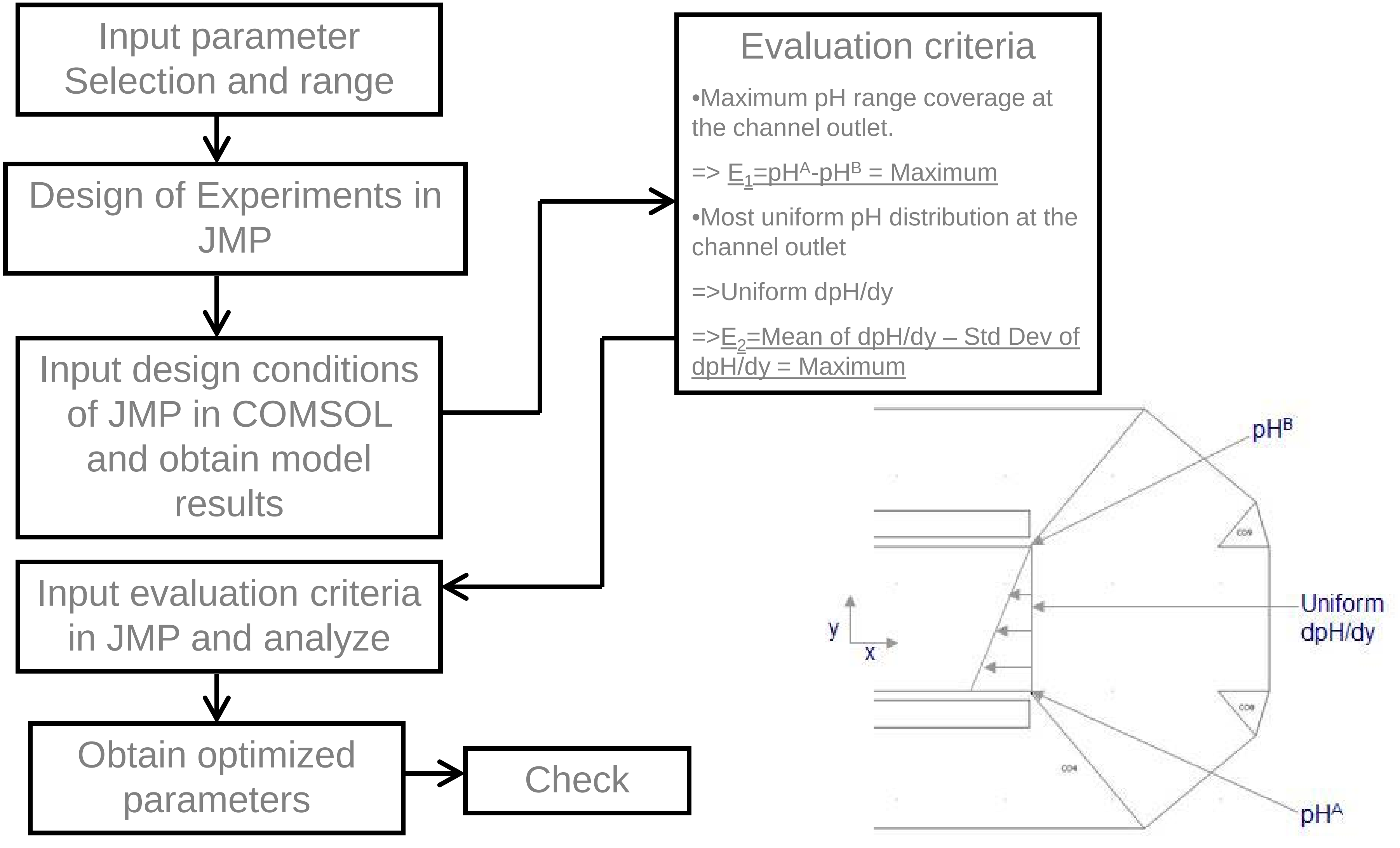
V, Electrode

Objectives:

-High pH gradient at the exit of microfluidic channel.

-Multiple chip configuration that can achieve pH resolution of 0.1

Design of Experiments Procedure



Input parameter Selection and range

Design of Experiments in JMP

Input design conditions of JMP in COMSOL and obtain model results

Input evaluation criteria in JMP and analyze

Obtain optimized parameters

Check

Evaluation criteria

- Maximum pH range coverage at the channel outlet.

=> $E_1 = pH^A - pH^B$ = Maximum

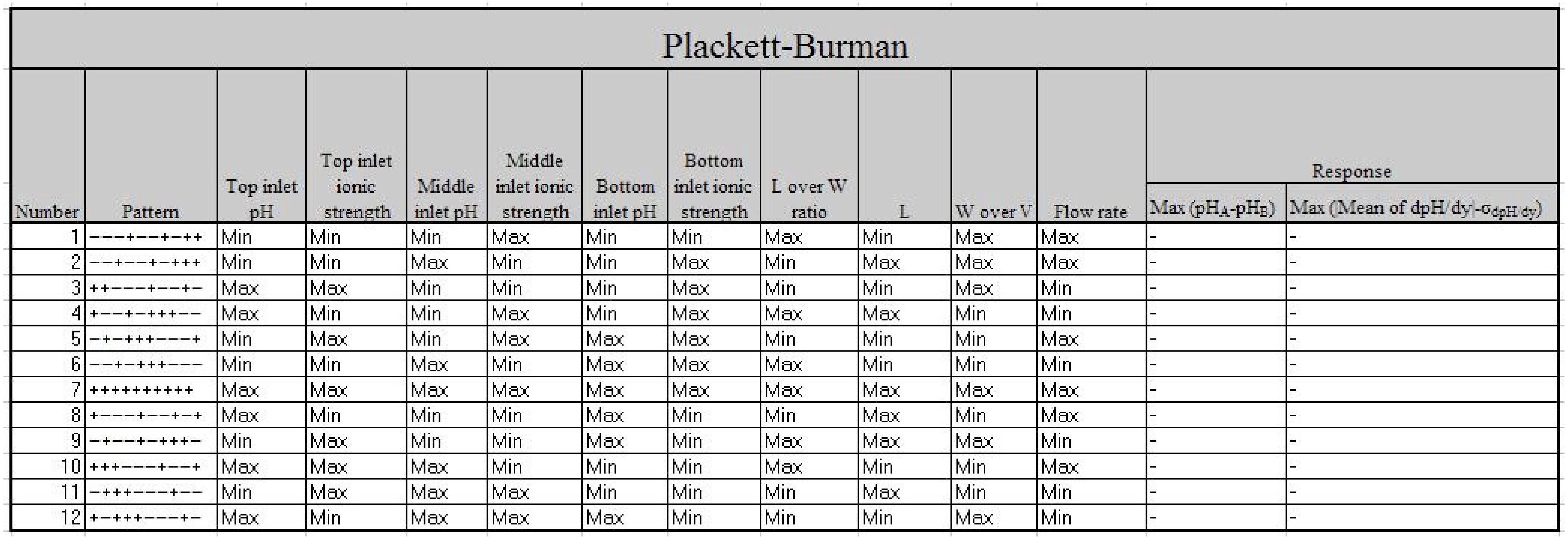
- Most uniform pH distribution at the channel outlet

=>Uniform dpH/dy

=>E_2=Mean of dpH/dy – Std Dev of dpH/dy = Maximum

Design of Experiments

Plackett-Burman 12 Runs



Plackett-Burman

Number	Pattern	Top inlet pH	Top inlet ionic strength	Middle inlet pH	Middle inlet ionic strength	Bottom inlet pH	Bottom inlet ionic strength	L over W ratio	L	W over V	Flow rate	Response	
												Max (pH_A-pH_B)	Max (Mean of dpH/dy|-$\sigma_{dpH/dy}$)
1	---+--+-++	Min	Min	Min	Max	Min	Min	Max	Min	Max	Max	-	-
2	--+--+-+++	Min	Min	Max	Min	Min	Max	Min	Max	Max	Max	-	-
3	++---+--+-	Max	Max	Min	Min	Min	Max	Min	Min	Max	Min	-	-
4	+--+-+++--	Max	Min	Min	Max	Min	Max	Max	Max	Min	Min	-	-
5	-+-+++---+	Min	Max	Min	Max	Max	Max	Min	Min	Min	Max	-	-
6	--+-+++---	Min	Min	Max	Min	Max	Max	Max	Min	Min	Min	-	-
7	++++++++++	Max	Max	Max	Max	Max	Max	Max	Max	Max	Max	-	-
8	+---+--+-+	Max	Min	Min	Min	Max	Min	Min	Max	Min	Max	-	-
9	-+--+-+++-	Min	Max	Min	Min	Max	Min	Max	Max	Max	Min	-	-
10	+++---+--+	Max	Max	Max	Min	Min	Min	Max	Min	Min	Max	-	-
11	-+++---+--	Min	Max	Max	Max	Min	Min	Min	Max	Min	Min	-	-
12	+-+++---+-	Max	Min	Max	Max	Max	Min	Min	Min	Max	Min	-	-

- R.L. Plackett and J.P. Burman proposed this method in 1946 in their famous paper ‘The Design of Optimal Multifactorial Experiments” in Biometrika (vol.33).
- This method is very economical and effective in understanding independent effect of each parameter.

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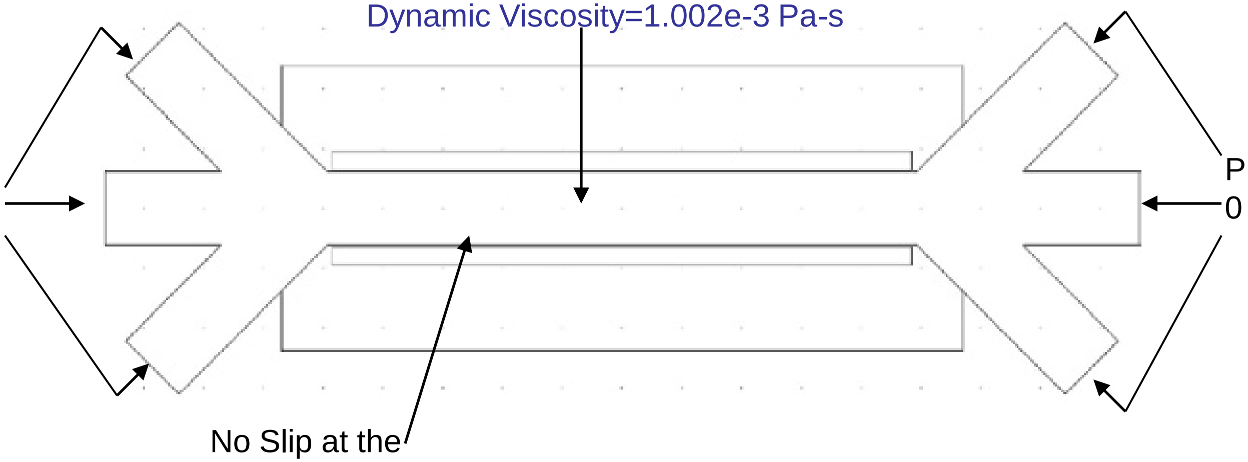
COMSOL Model Set-up

Incompressible Navier-Stokes

$$\rho\frac{\partial \mathbf{u}}{\partial t} - \nabla\cdot[\eta(\nabla\mathbf{u} + (\nabla\mathbf{u})^T)] + \rho(\mathbf{u}\cdot\nabla)\mathbf{u} + \nabla p = \mathbf{F}$$

$$\nabla\cdot\mathbf{u} = 0$$



Density=998 kg/m3
Dynamic Viscosity=1.002e-3 Pa-s

Inflow Velocity

Pressure
0 Pa

No Slip at the
Wall

🟦 Subdomain Setting

⬛ Boundary Setting

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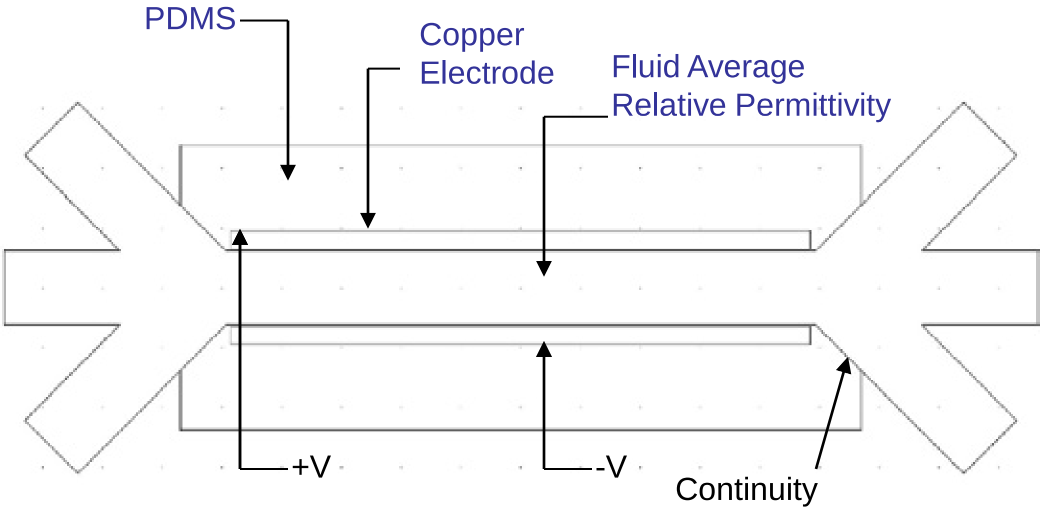
COMSOL Model Set-up

Electrostatics

$$\mathbf{D} = \varepsilon_0(1+\chi_e)\mathbf{E} = \varepsilon_0\varepsilon_r\mathbf{E}$$



🟦 Subdomain Setting

⬛ Boundary Setting

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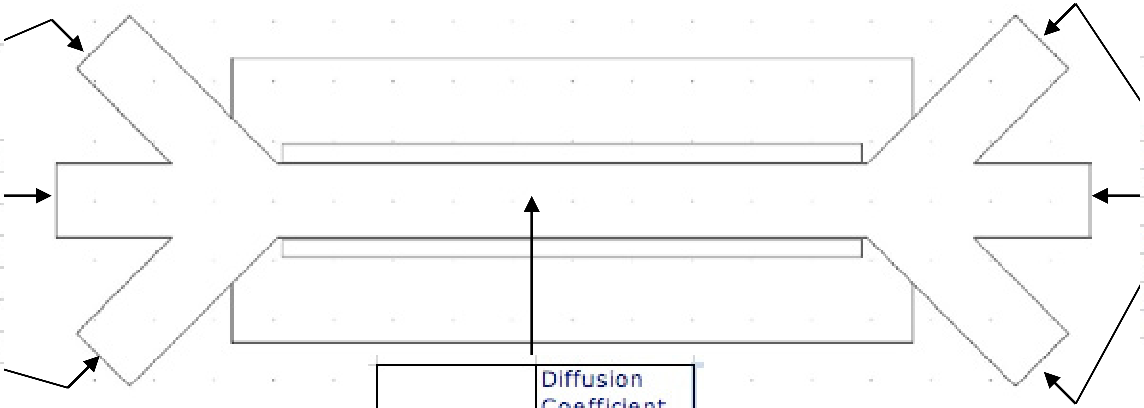
COMSOL Model Set-up

Electrokinetics

$$\frac{\partial c_i}{\partial t} + \nabla \cdot (-D_i \nabla c_i - z_i u_{\mathrm{m}i} F c_i \nabla V + c_i \mathbf{u}) = R_i$$



Solute Concentration
H+
TrisNeutral
Tris+
Cl-
Na+
Acetate-
Acetic Acid

Solute	Exit Flux
H+	-ntflux_c_chekf
TrisNeutral	-ntflux_c_tn_chekf
Tris+	-ntflux_c_tp_chekf
Cl-	-ntflux_c_cl_chekf
Na+	-ntflux_c_na_chekf
Acetate-	-ntflux_c_ace_chekf
Acetic Acid	-ntflux_c_acid_chekf

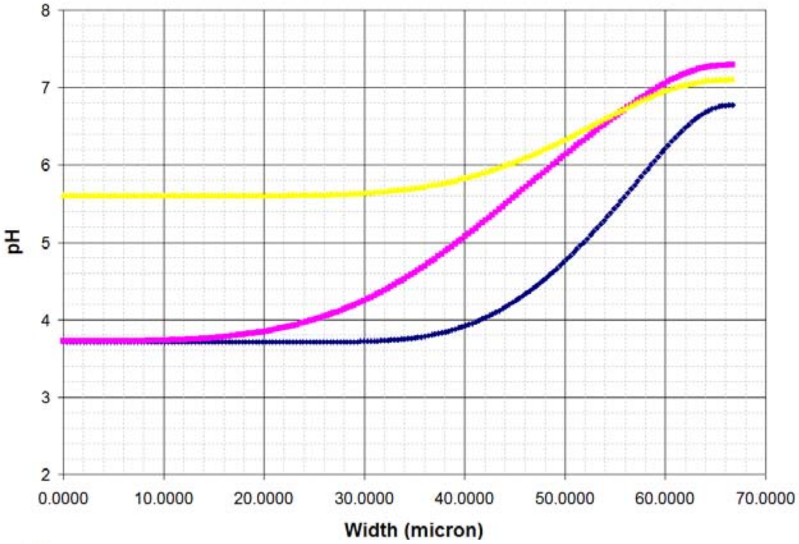
Solute	Diffusion Coefficient (m2/s)
H+	9.30E-09
TrisNeutral	1.50E-09
Tris+	1.50E-09
Cl-	9.00E-08
Na+	1.33E-09
Acetate-	1.50E-09
Acetic Acid	1.50E-09

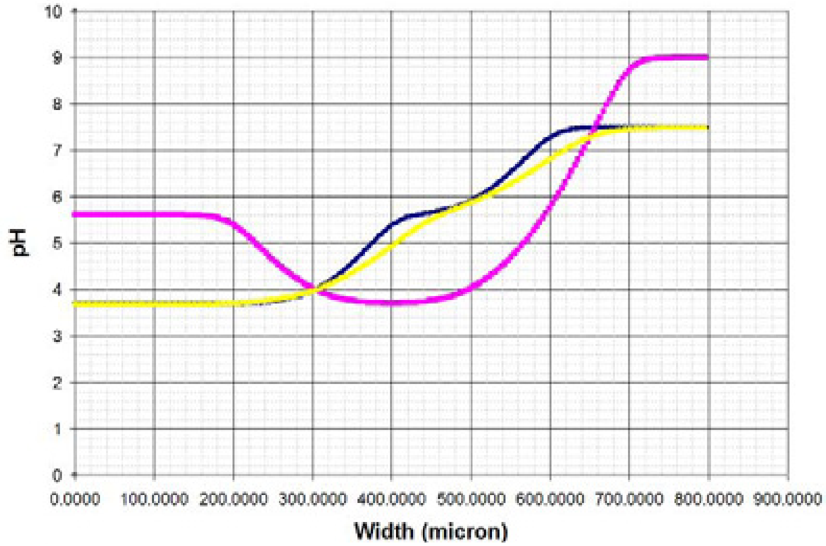
🟦 Subdomain Setting

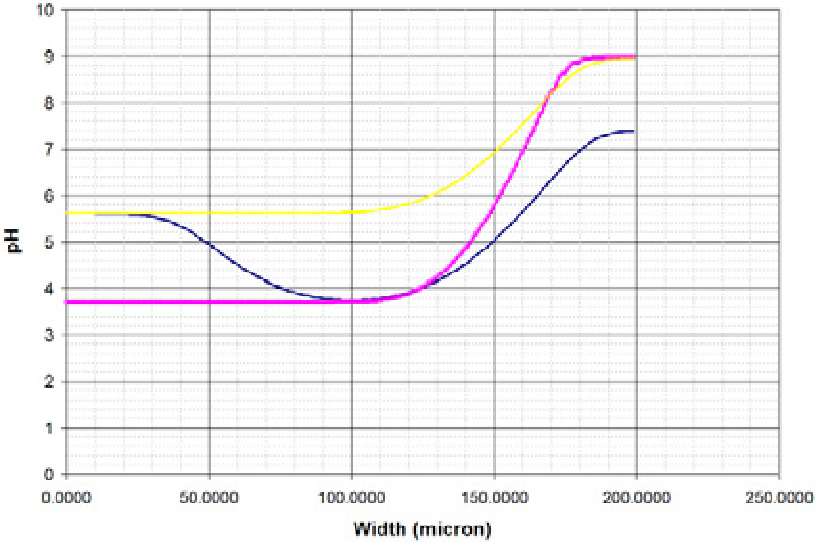
⬛ Boundary Setting

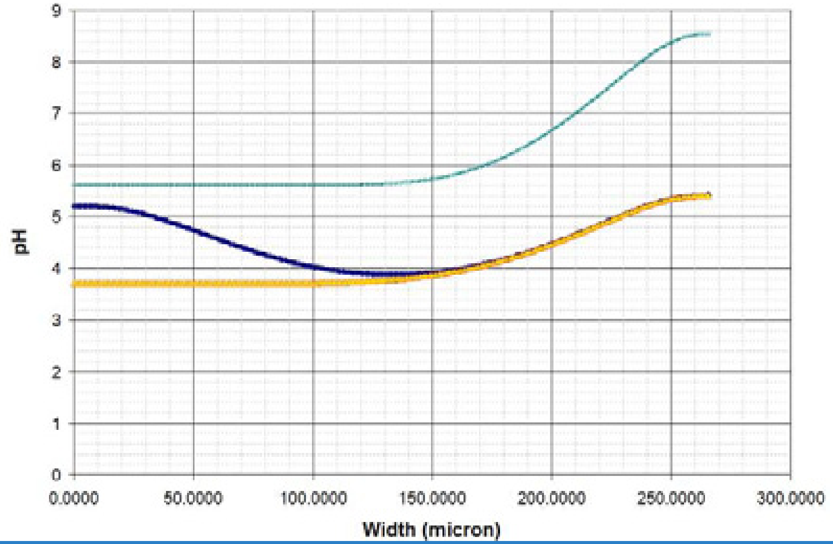
COMSOL Results

pH Distribution at the Exit of the Channel





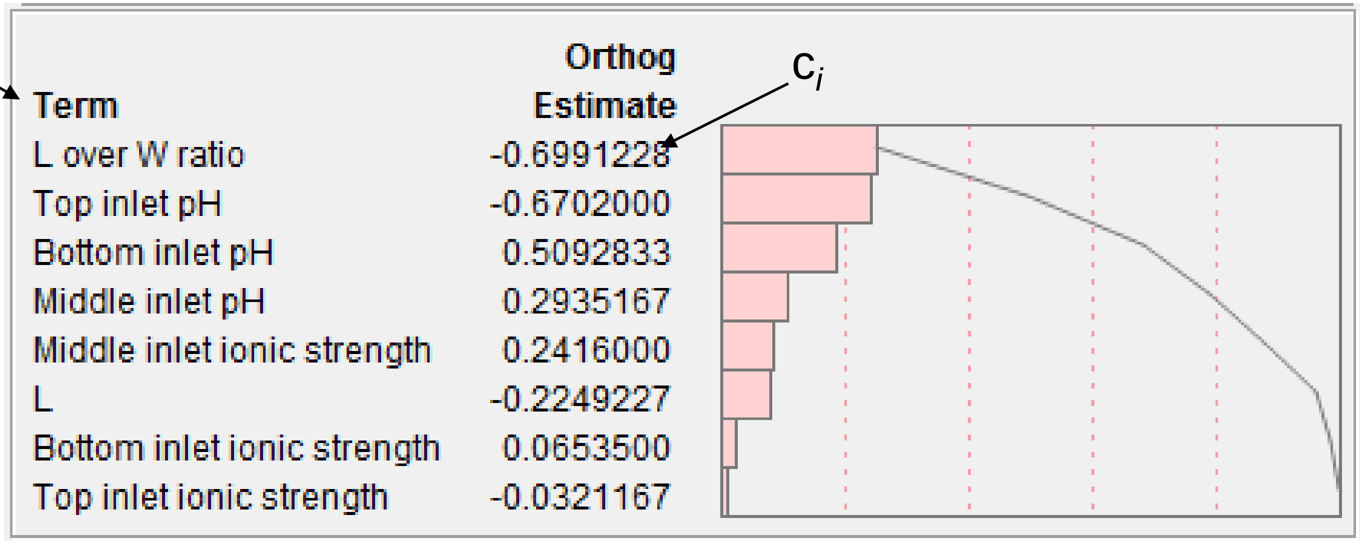




Statistical Optimization from COMSOL Results: Max pH

$$f_1=\Sigma_{(i=0,10)}(c_i*p_i)$$

where f_1=Maximum value of ΔpH,
c_i=constant, p_i=parameter



p_i

c_i

Term	Orthog Estimate
L over W ratio	-0.6991228
Top inlet pH	-0.6702000
Bottom inlet pH	0.5092833
Middle inlet pH	0.2935167
Middle inlet ionic strength	0.2416000
L	-0.2249227
Bottom inlet ionic strength	0.0653500
Top inlet ionic strength	-0.0321167

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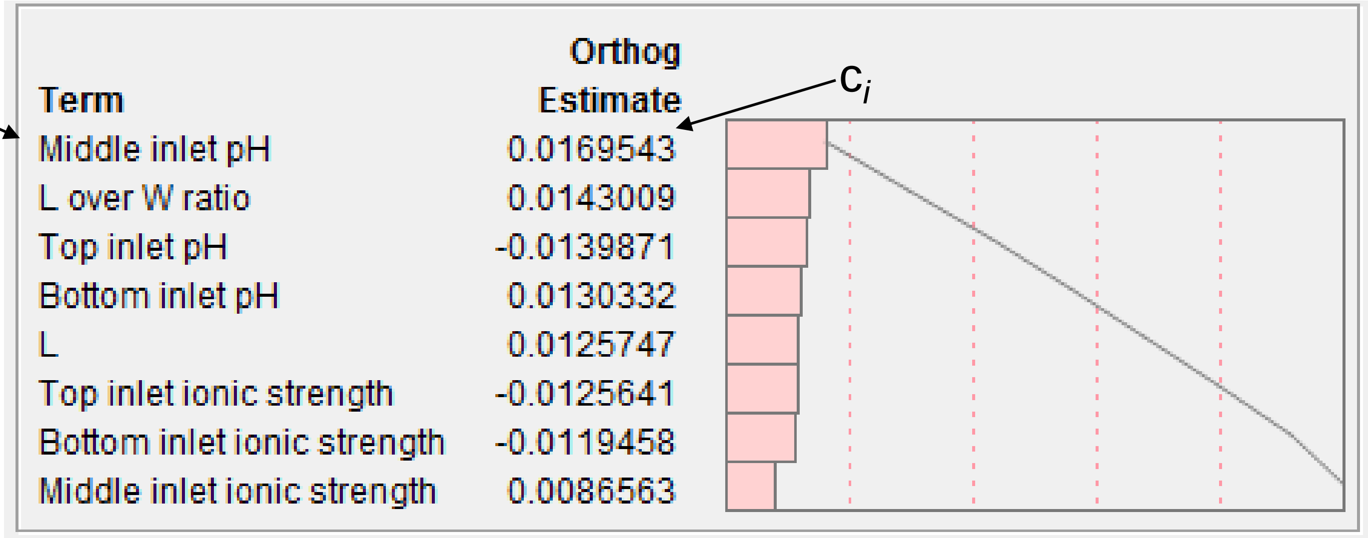
Statistical Optimization from COMSOL Results: Uniform pH

$f_2=\Sigma(i=0,10)(c_i*p_i)$

where f_2=Maximum pH Uniformity,
c_i=constant, p_i=parameter

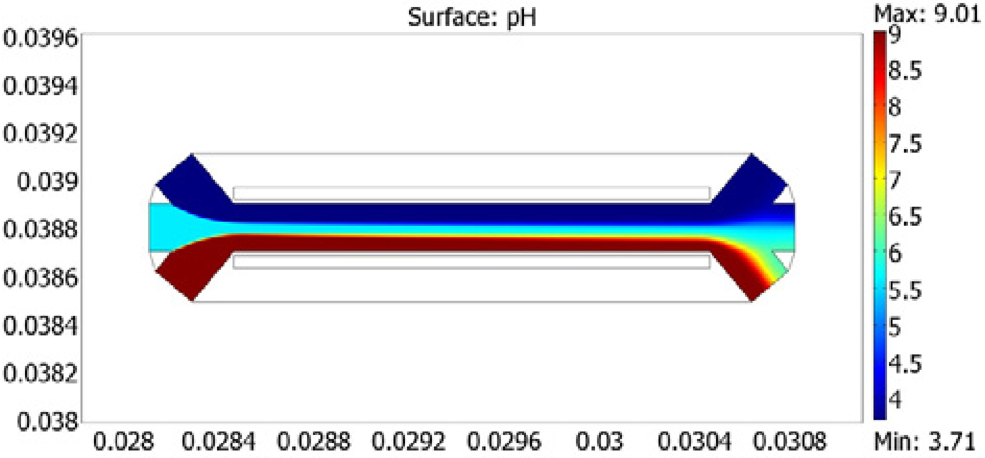
p_i

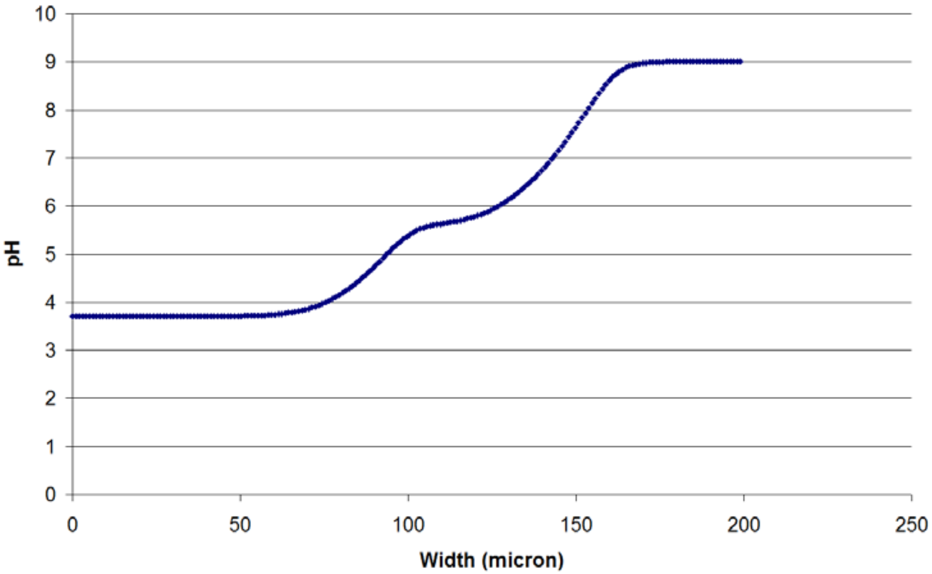
c_i



Term	Orthog Estimate
Middle inlet pH	0.0169543
L over W ratio	0.0143009
Top inlet pH	-0.0139871
Bottom inlet pH	0.0130332
L	0.0125747
Top inlet ionic strength	-0.0125641
Bottom inlet ionic strength	-0.0119458
Middle inlet ionic strength	0.0086563

COMSOL Results based on Statistical Analysis: No Potential Applied





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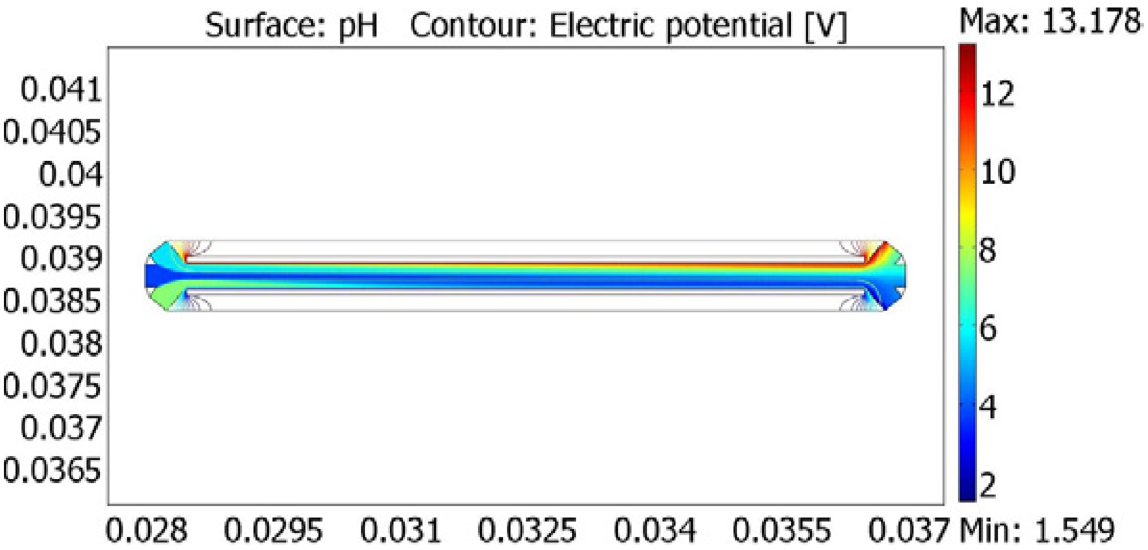
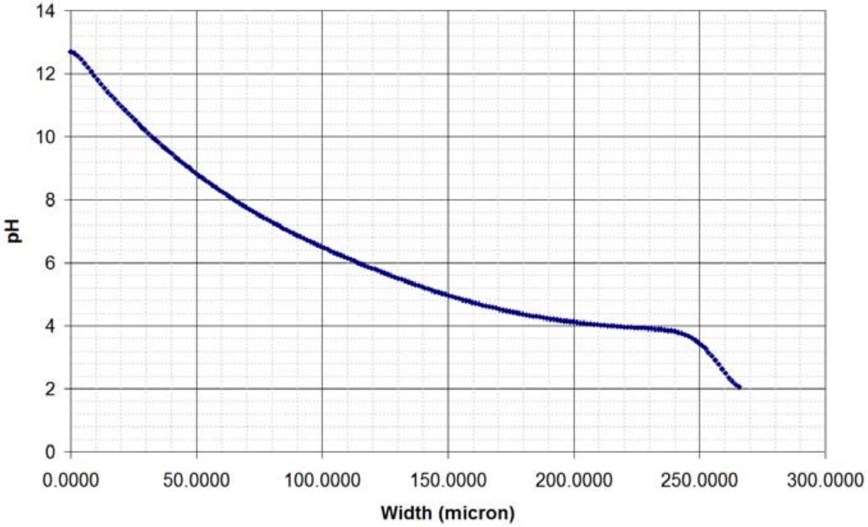
COMSOL Results based on Statistical Analysis: Finite Potential Applied





Conclusions

- Most dominant parameters controlling the process are identified.
- An optimized design is proposed by numerical modeling.
- pH gradient of range 1.5-13 with high uniformity is achieved.

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Future Work

- Experimental Validation.
- Run DOE with more combination of few important parameters to fine tune the current design.
- Continue similar DOE for subsequent channels until a resolution of 0.1 is achieved.

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