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On-Chip Aqueous Two-Phase Extraction for Protein Isolation from Cell Lysate

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Motivation

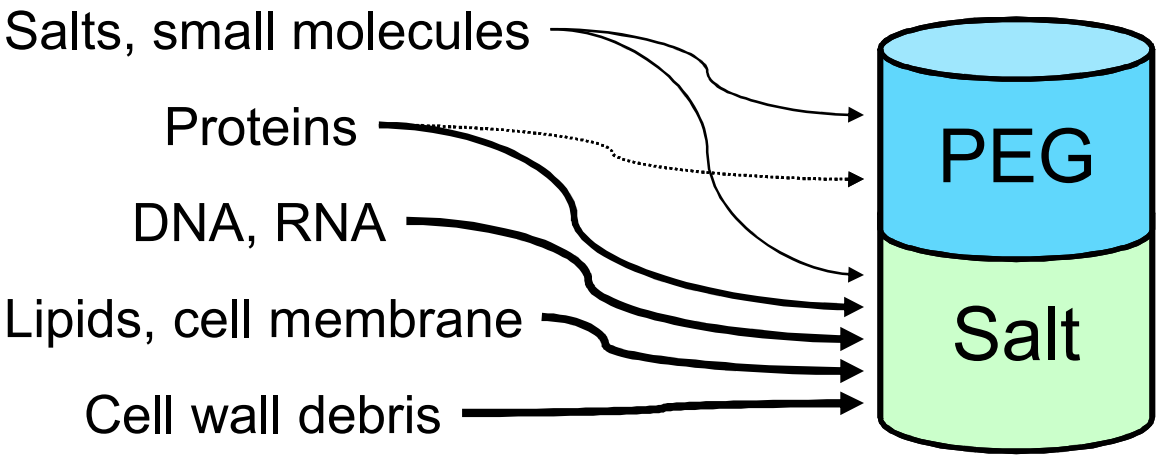
- Reduced reagent usage and sample consumption is a major driver of microfluidics research
- Several examples of microliter-scale or smaller cell cultures
- Numerous examples of sensitive protein analyses on chip
  - Microchannel electrophoresis, chromatography, immunoassays, interface to mass spectrometry
- Tools are needed to scale down the intermediate stages of processing, between cell culture and analyses.
  - On-chip cell lysis by chemical, mechanical, and electrical means are currently being developed.
  - Processes for micro-scale isolation of desired proteins are still needed.

Aqueous Two-Phase Systems

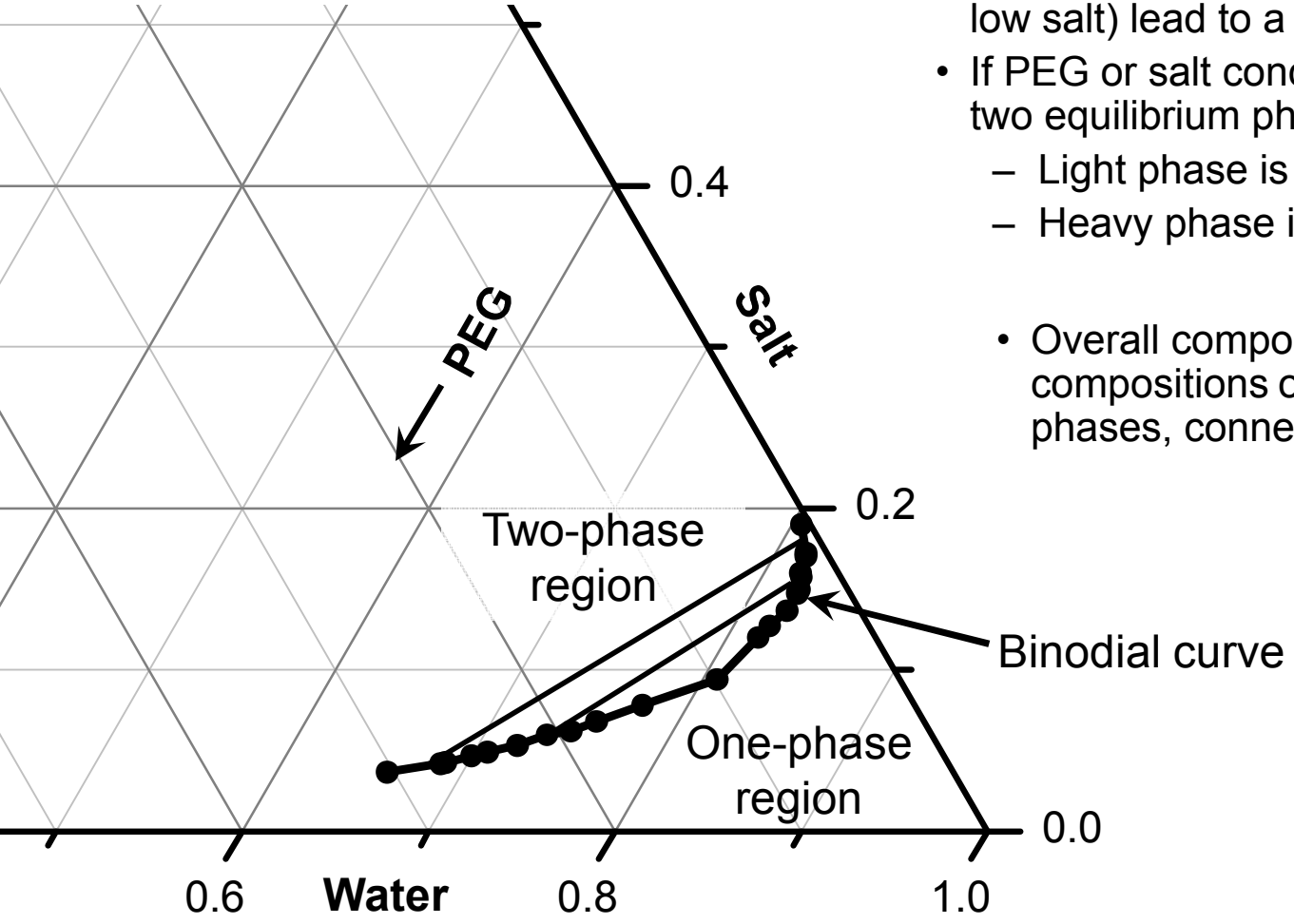
- Many mixtures of polymers and/or salts form multiphase mixtures
  - PEG/dextran
  - PEG/salts
  - Many, many others – up to 17-phase systems have been created
  - Composition of both phases is mostly water – mild and easy for proteins to tolerate
  - Very low interfacial tension

Aqueous Two-phase extraction

- Has been used industrially on a very large scale for purification of a wide variety of different recombinant proteins, enzymes, etc.
- Systems are generally mild, preserving protein structure
- Partitioning of desired components can often be tailored by carefully choosing phase system.
- In a PEG-Potassium Phosphate two-phase system:
  - Many undesired components will partition to the salt phase
  - Proteins vary greatly in their partitioning, but many tend to partition to the salt phase
  - Some proteins partition very strongly to the PEG phase, and can be isolated from other cell components in a single step.



Thermodynamics of Aqueous Two-Phase Mixtures



- Some overall compositions (e.g. low PEG, low salt) lead to a single phase
- If PEG or salt concentration is high enough, two equilibrium phases form spontaneously
  - Light phase is PEG-rich
  - Heavy phase is salt-rich

- Overall composition determines compositions of both light and heavy phases, connected by tie lines

Wohlfarth, CRC Handbook of Thermodynamic Data of Aqueous Polymer Solutions

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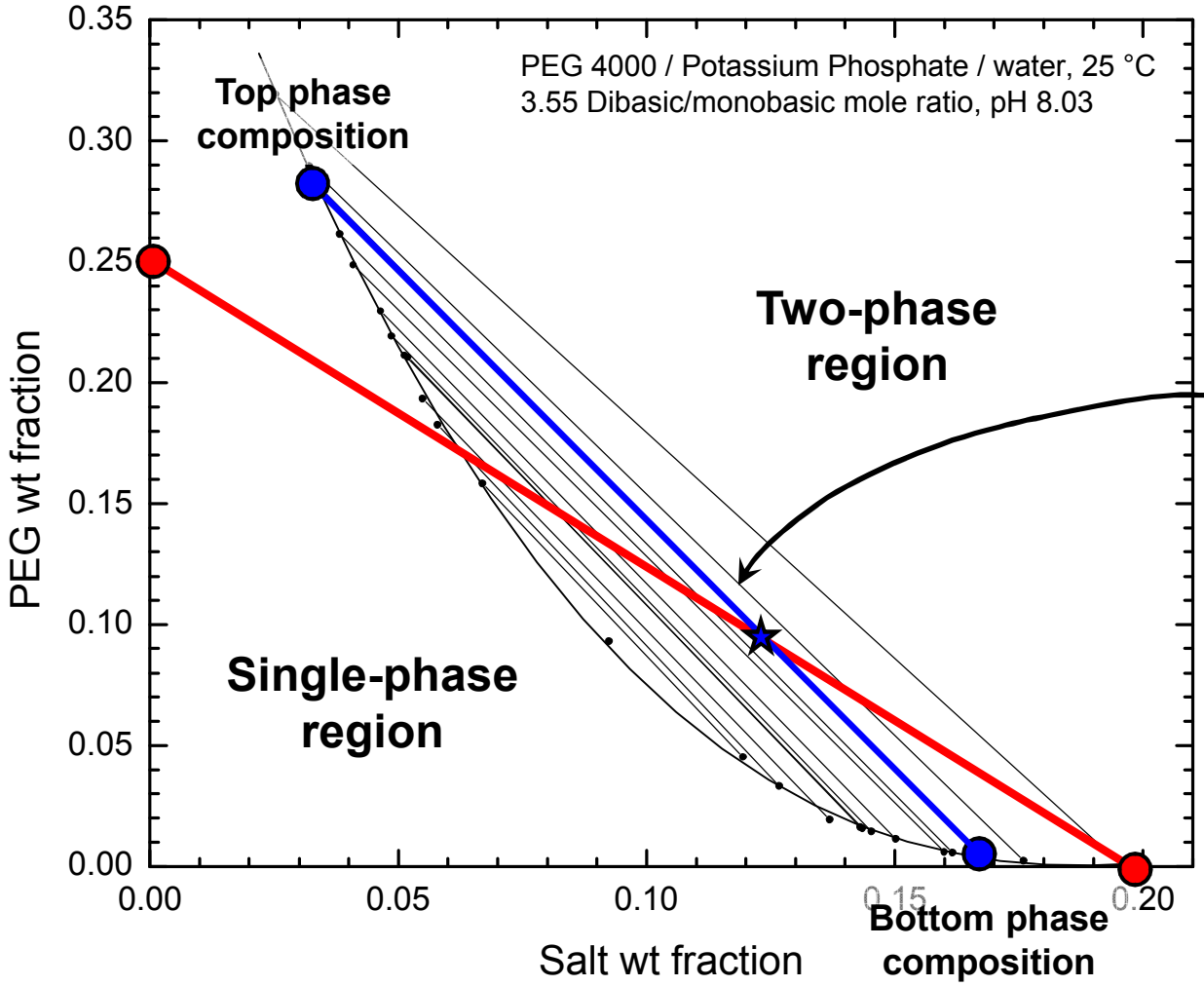
PEG-Salt Phase Diagram



Tie Line Length is important in determining partitioning

Wohlfarth, *CRC Handbook of Thermodynamic Data of Aqueous Polymer Solutions*

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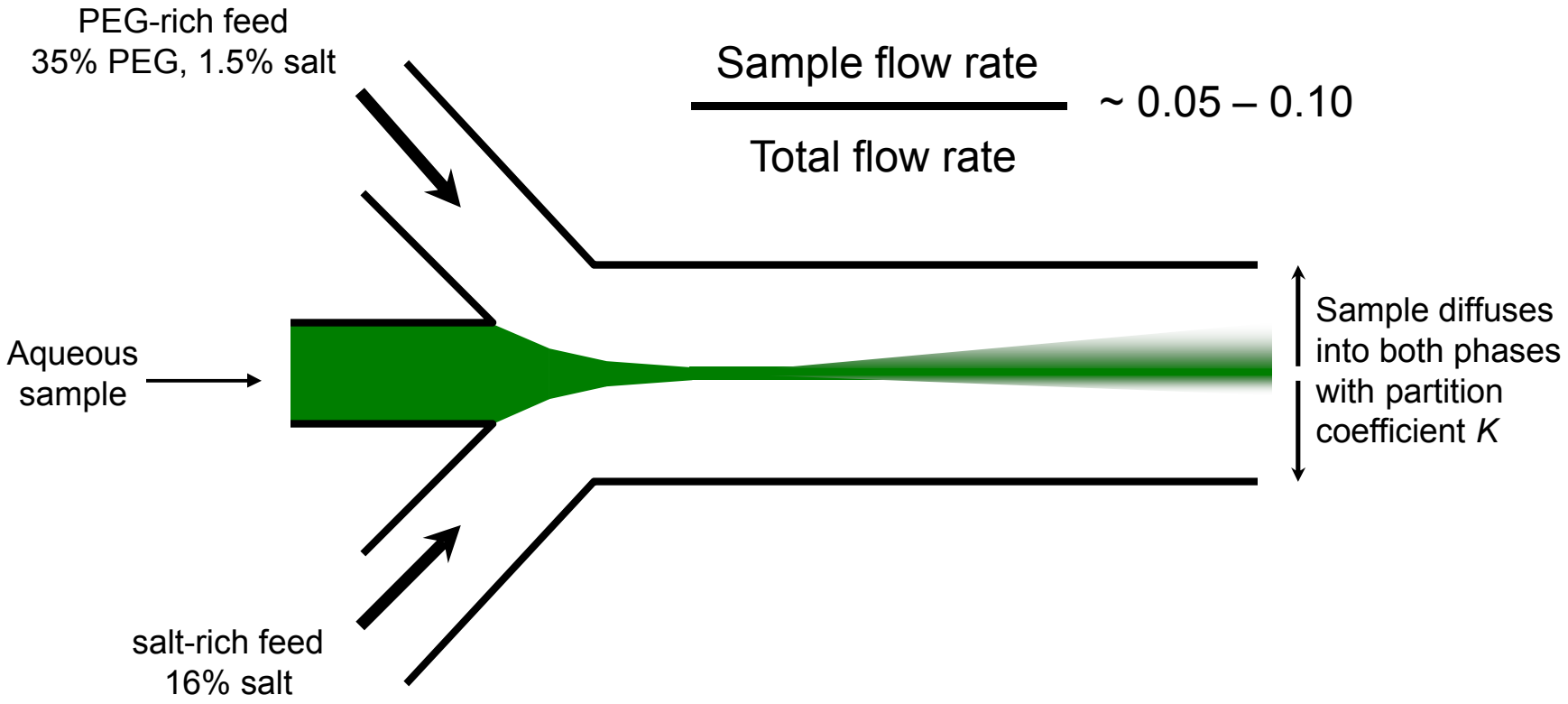
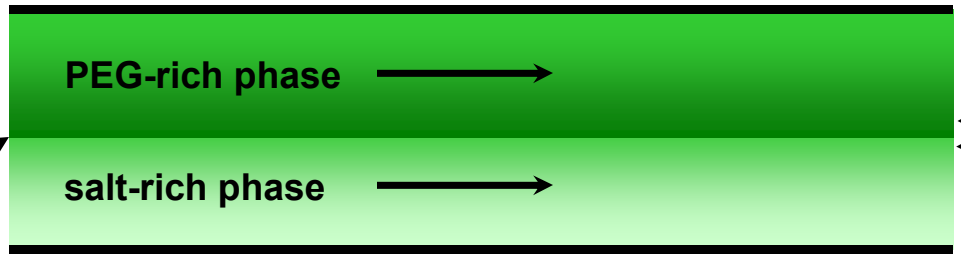
Flow Configuration



Far downstream…



stable
interface

Equilibrium at interface

$$c_{i,PEG} = K c_{i,salt}$$

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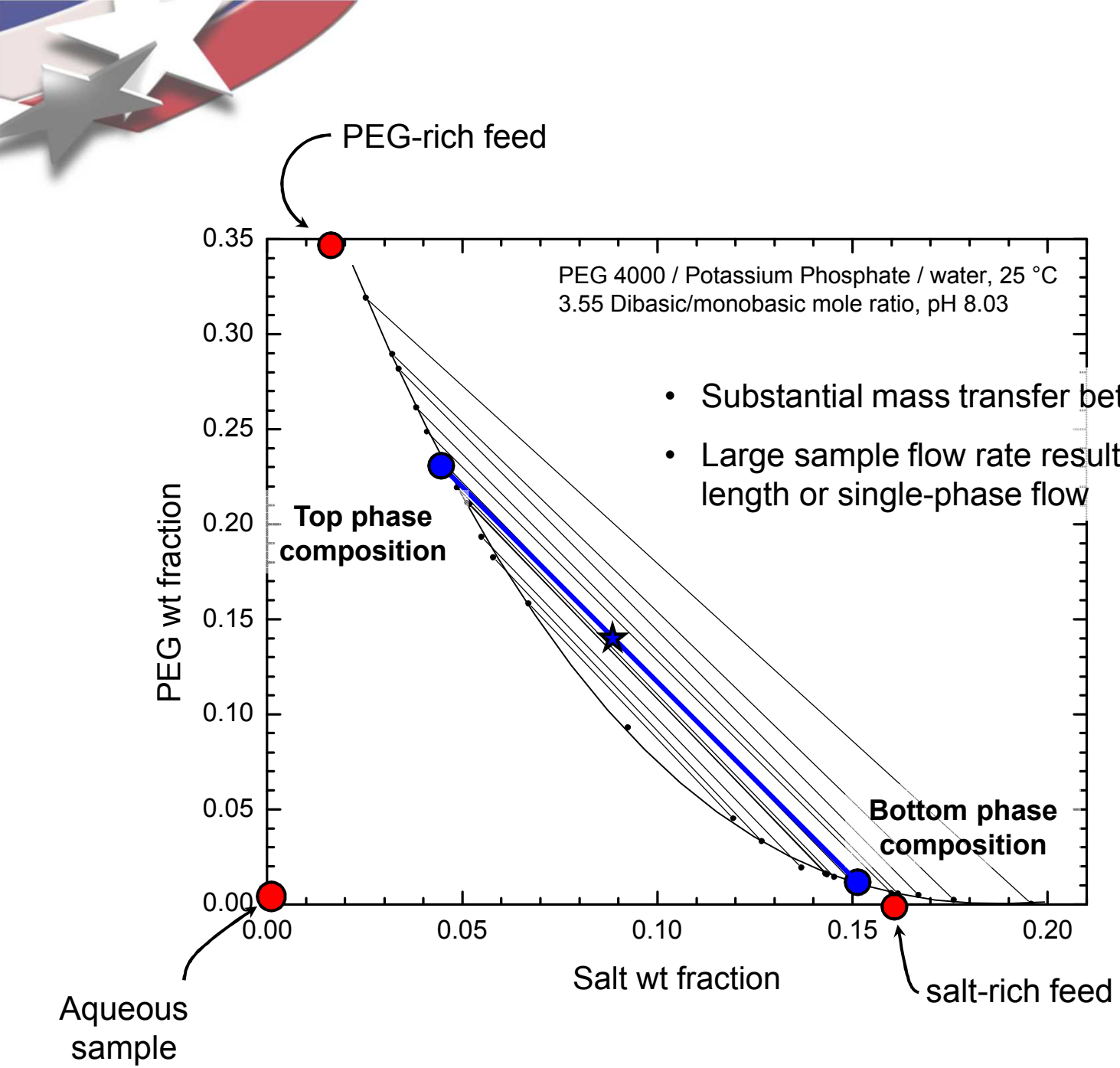

- Substantial mass transfer between phases
- Large sample flow rate results in short tie line length or single-phase flow

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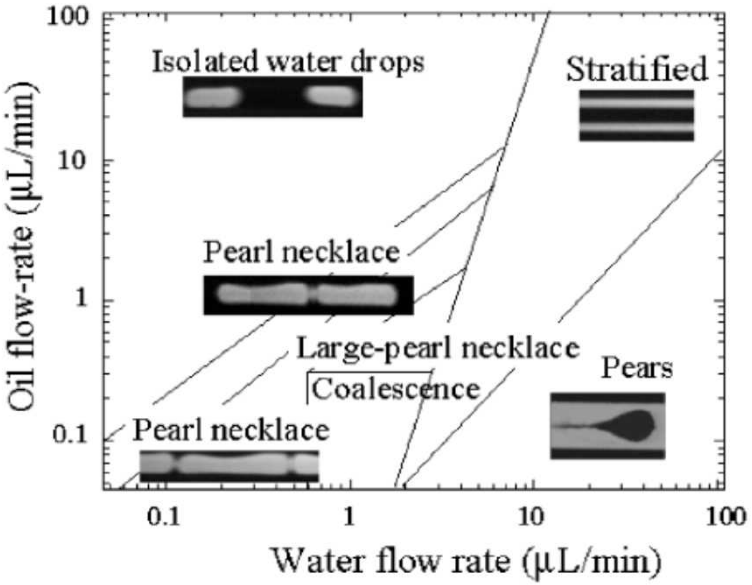
Extraction in microfluidic chips

- There are several reports of aqueous/organic extraction and stratified flow in microfluidic chips
  - In oil/water systems, the pure components are nearly immiscible in each other (very small single-phase region)
  - *Ca* = $\mu U / \gamma$ is important parameter for determining flow characteristics
  - High interfacial tension (~10-50 mN/m) leads to complex flow behavior
  - Surfactants help to achieve side-by-side or stratified flow

Dreyfus *et al*, *Phys. Rev. Lett.* 2003
Hexadecane and water with SPAN 80



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Aqueous Two-Phase Systems in Microfluidic Chips

- Very low interfacial tension (1-100 μN/m) makes stratified flow easy to achieve
  - Regimes with droplets can occur
- A few examples in the literature of ATPS in microfluidic devices for protein or cell fractionation

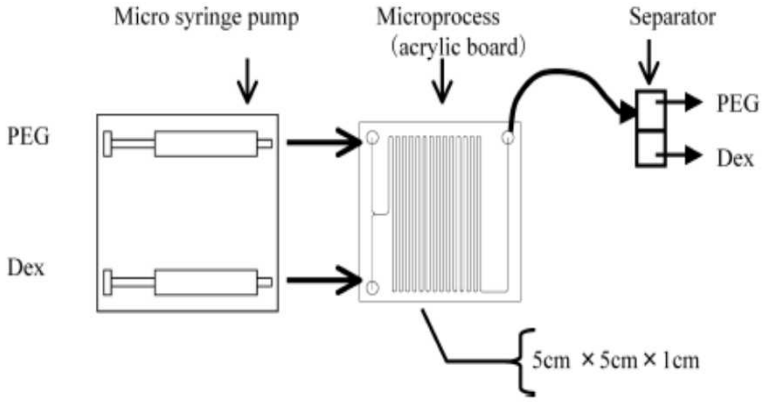
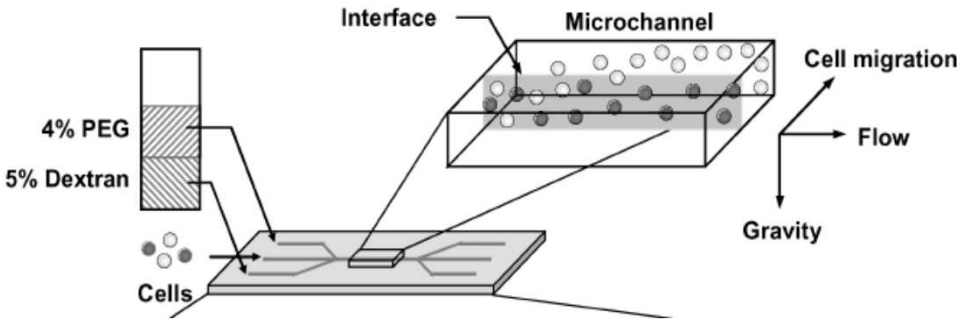


Fig. 2 Whole image of microflow aqueous polymer two-phase system

Asami *et al.*, *Kagaka Kogaku Runbunshu* 2004



Nam *et al.*, *Biomed. Microdev.* 2005
(Fractionation of live/dead cells)

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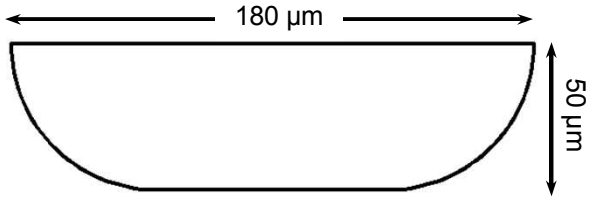
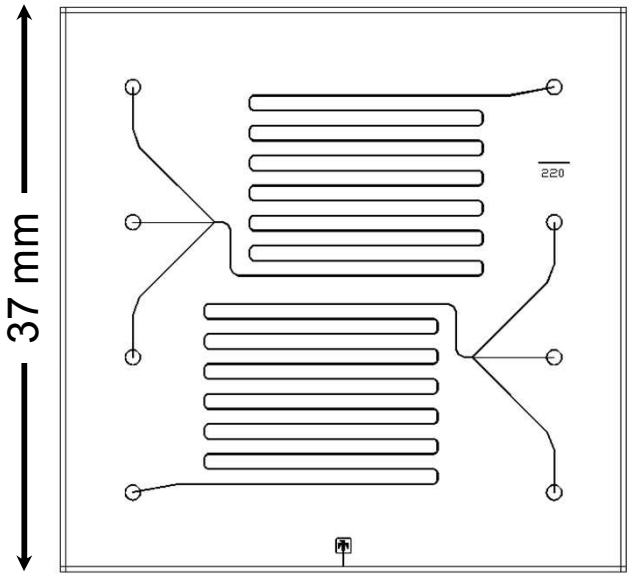
Initial channel designs

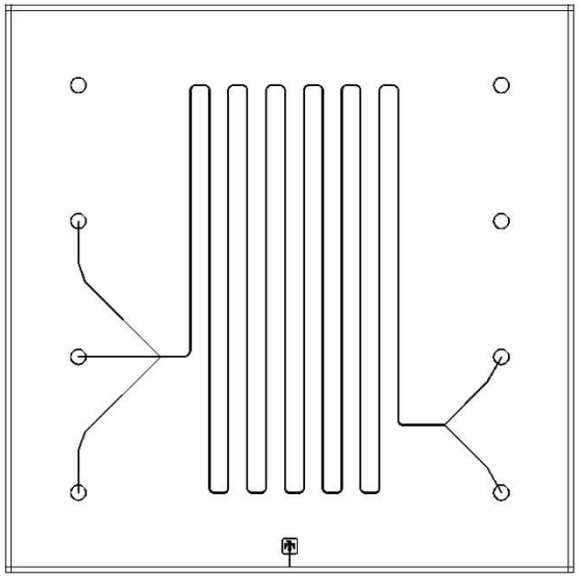
- Glass chips for initial studies
- Long, serpentine channels for long residence time in small footprint
- Relatively large diameter keeps pressure drop manageable
- Channels are covalently coated with polyacrylamide or poly-*N*-hydroxyethylacrylamide to prevent protein adsorption and unusual flow patterns

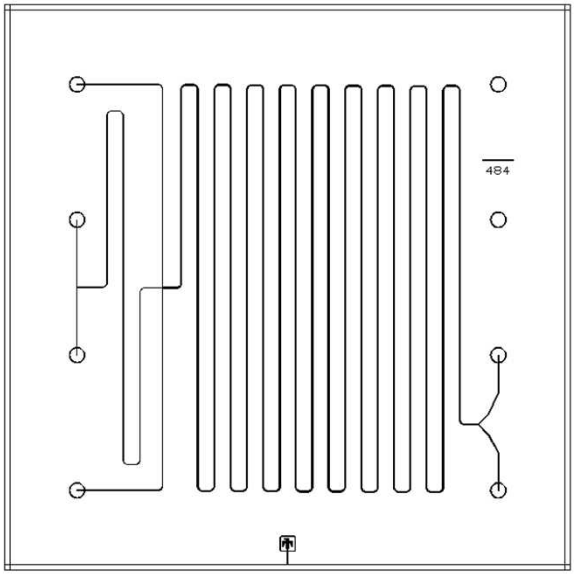


Channel cross section



220 mm channel


326 mm channel



484 mm channel

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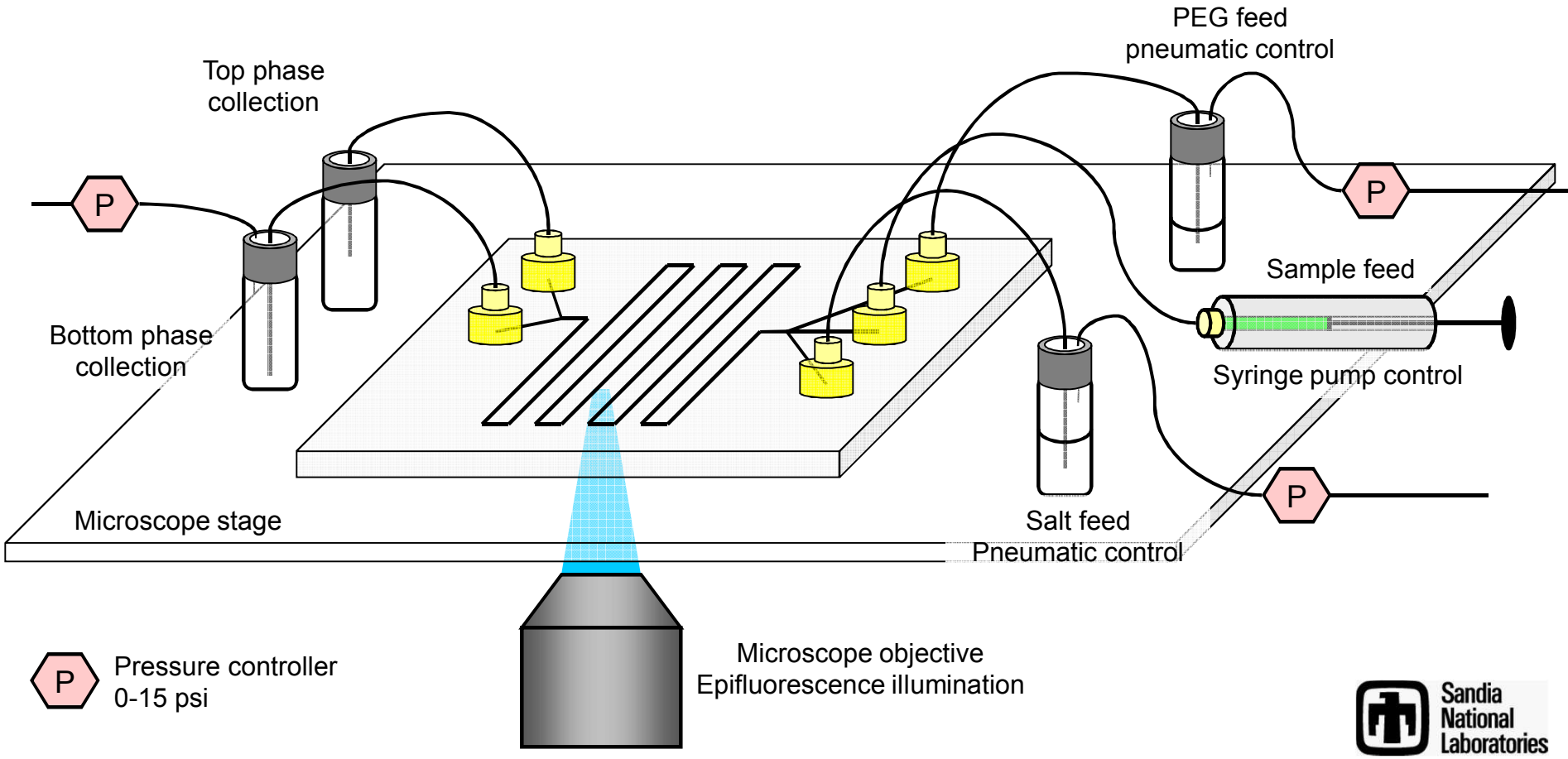
Experimental Setup

One possible configuration – other setups are possible!



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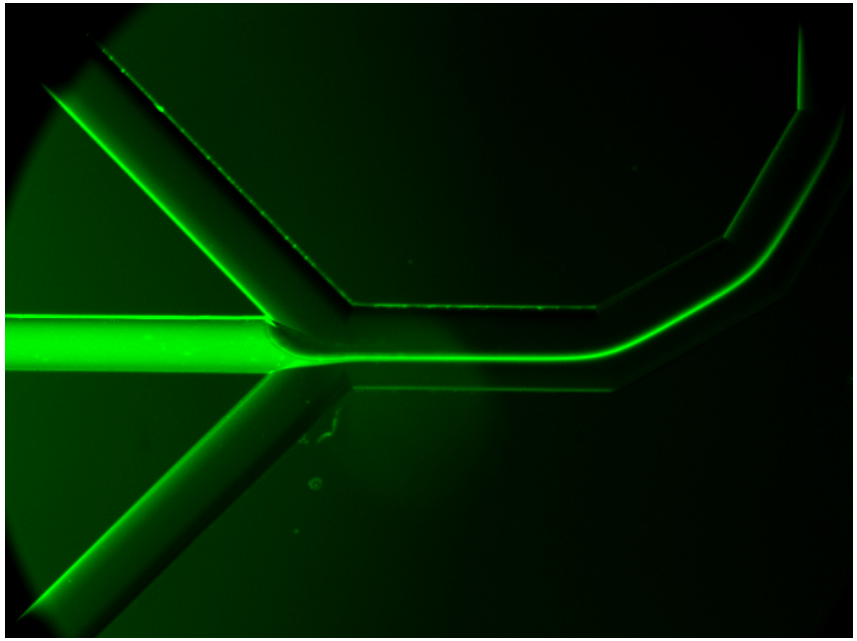
Stability of interface

- Video with FITC going along entire length of channel



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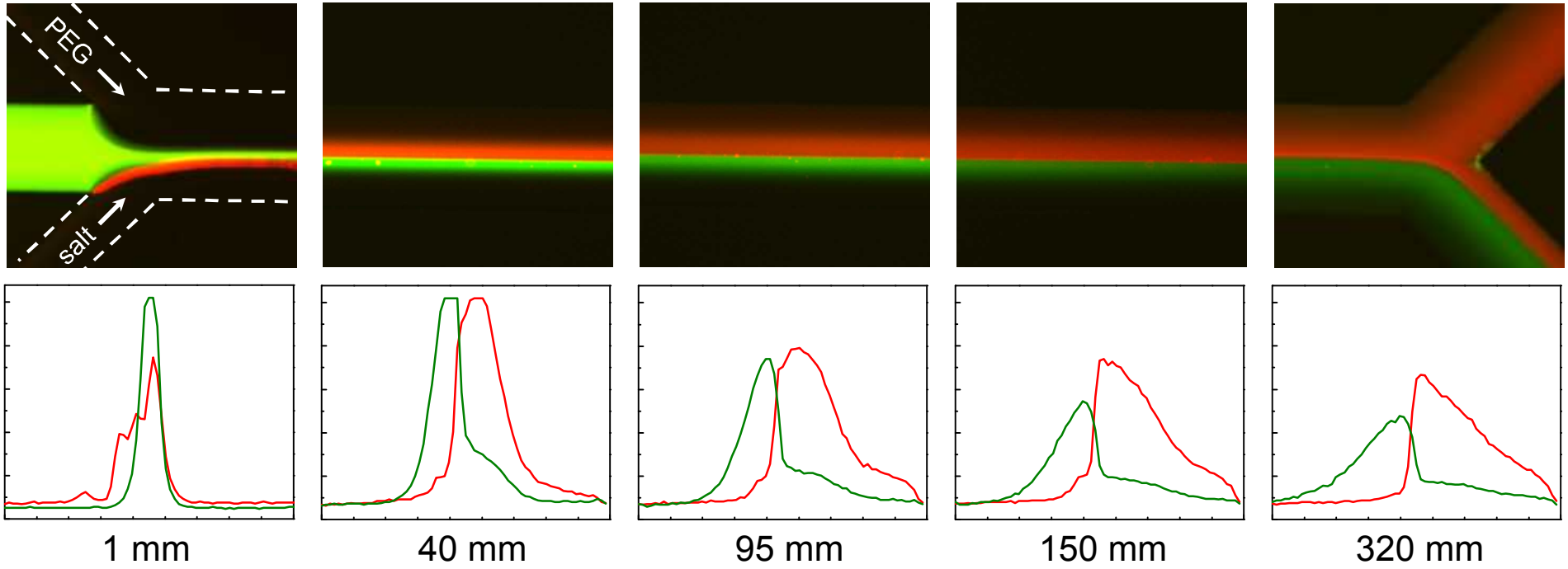
Partitioning of Proteins

- Example – fluorescently labeled BSA (green) and β-galactosidase (red) fed simultaneously
  - ΔP ~ 13.5 psi, Total flow rate ~ 7 µl/min (15 mm/s)
  - BSA partitions to salt phase; β–gal partitions strongly to PEG



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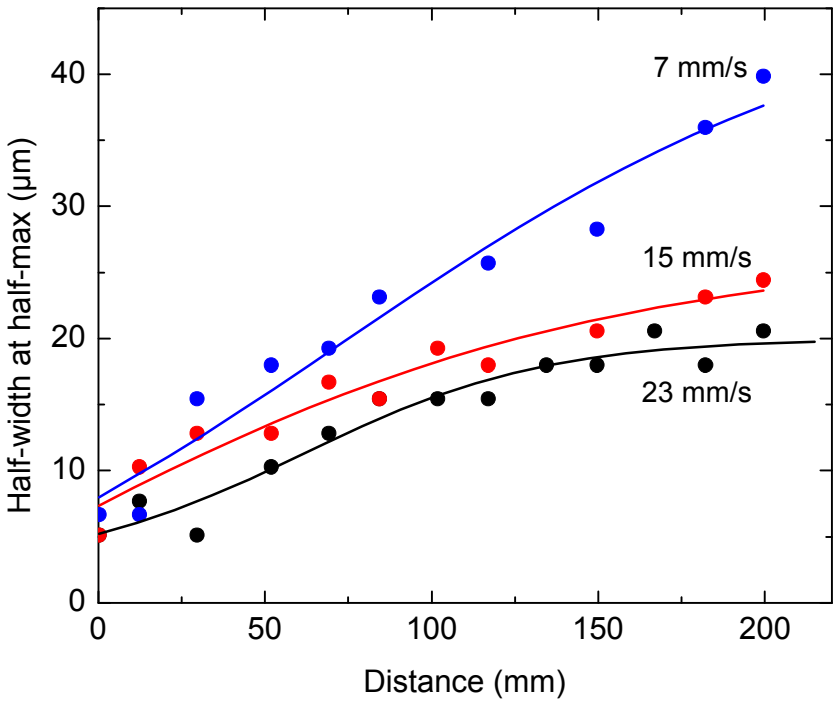
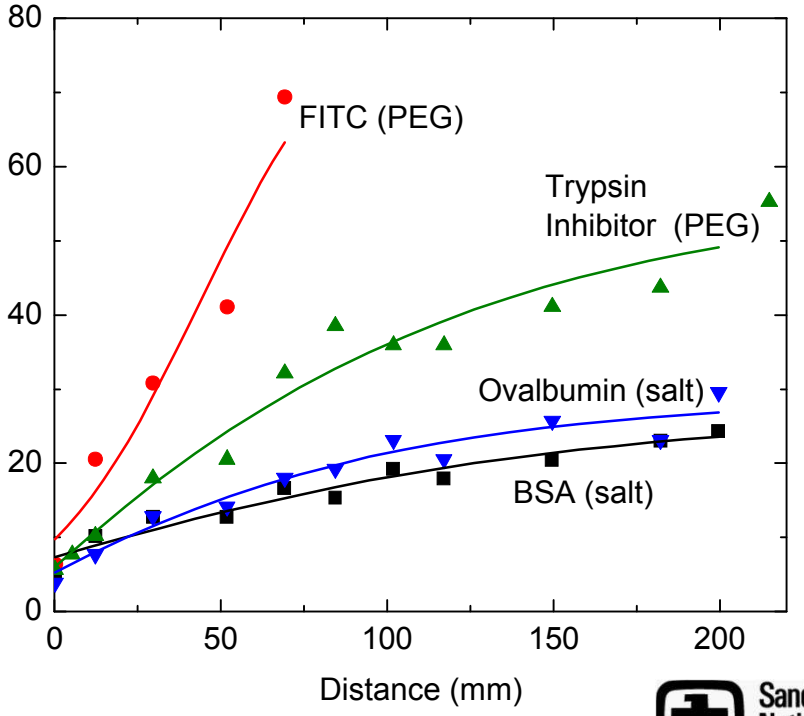
Spreading of sample from interface

- Equilibration of sample concentration across the channel depends on flow rate and diffusivity of analyte
- Diffusivity may be different in PEG and salt phases

Spreading of BSA on salt-rich side as a function of velocity



Spreading of different analytes at 15 mm/s



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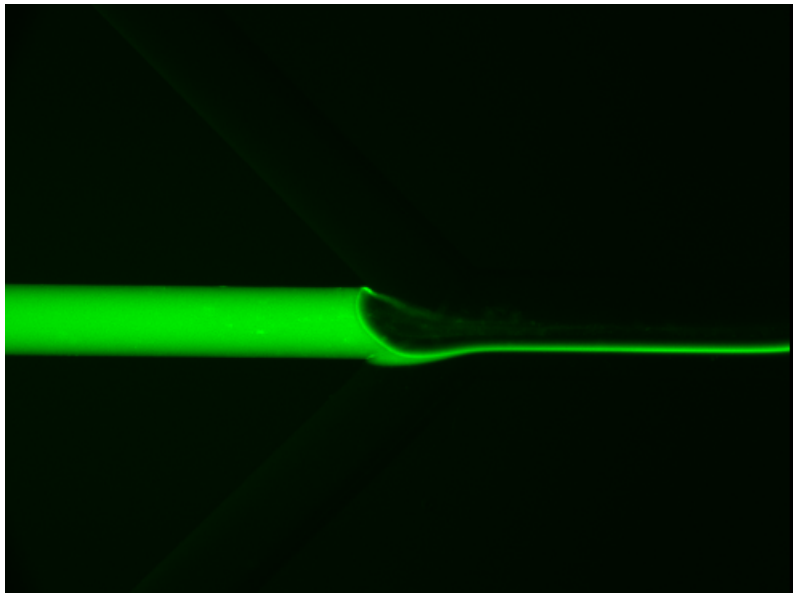
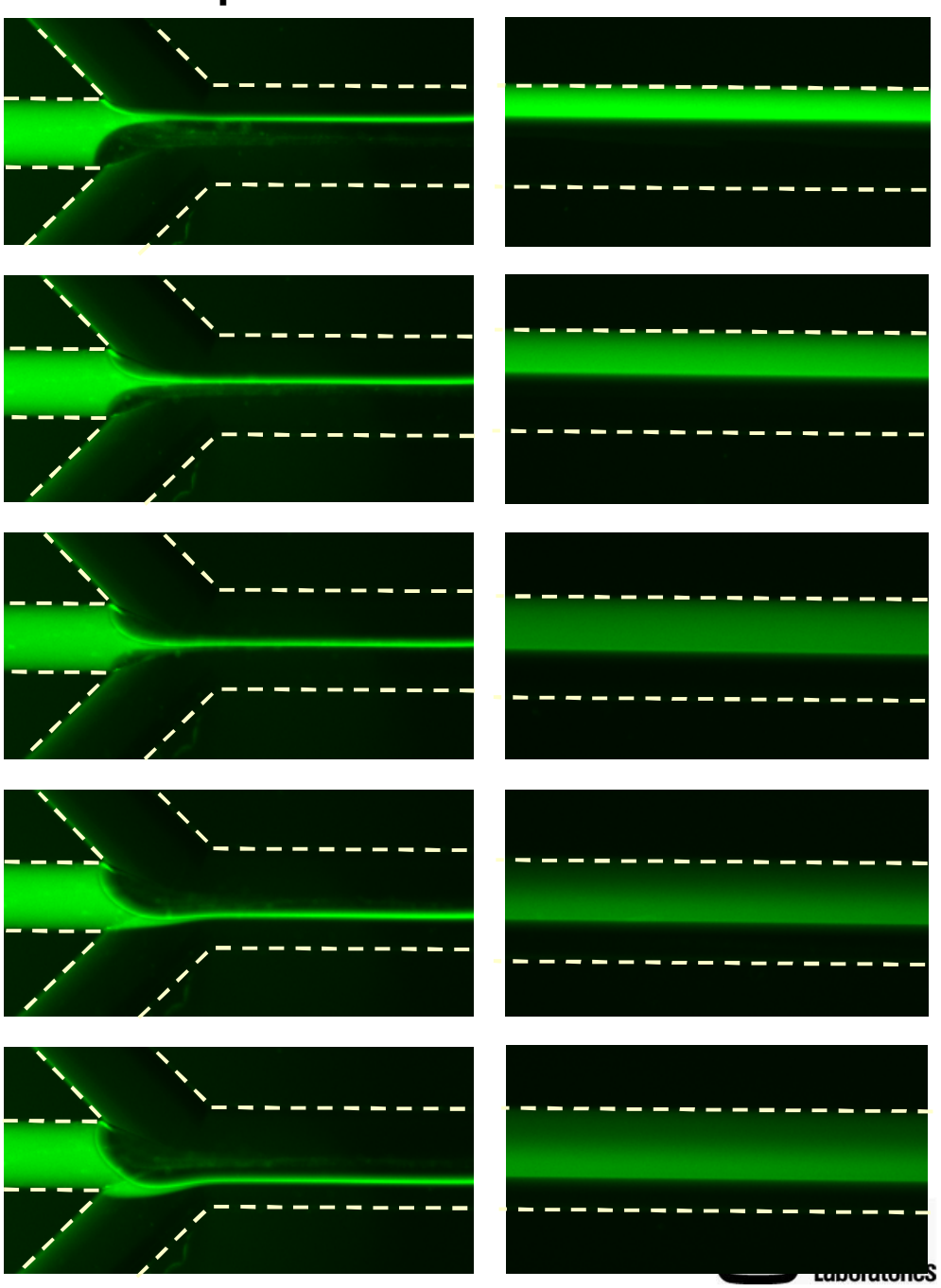
- Feed ratio of PEG and salt determines position of the interface
- Volume ratio of phases affects equilibrium amount of sample recovered in each phase

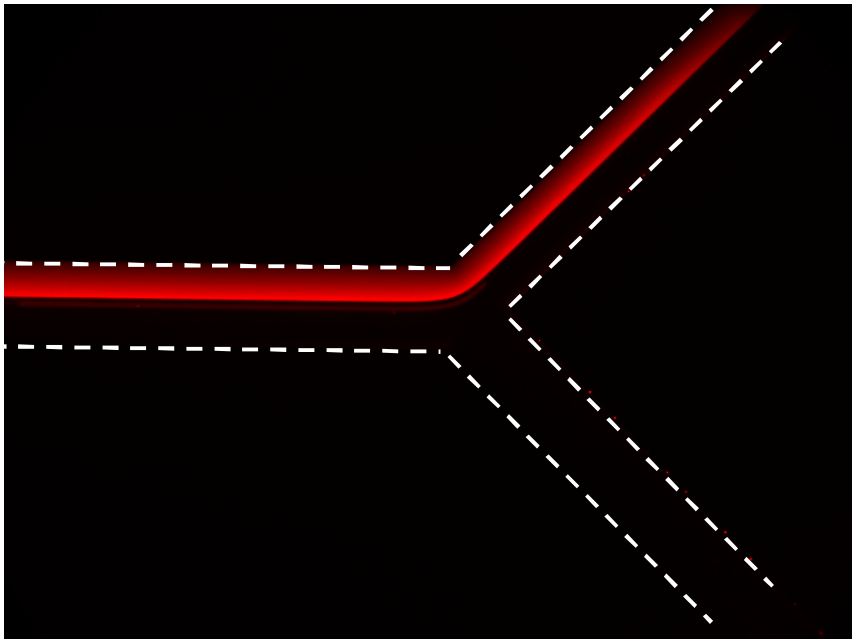




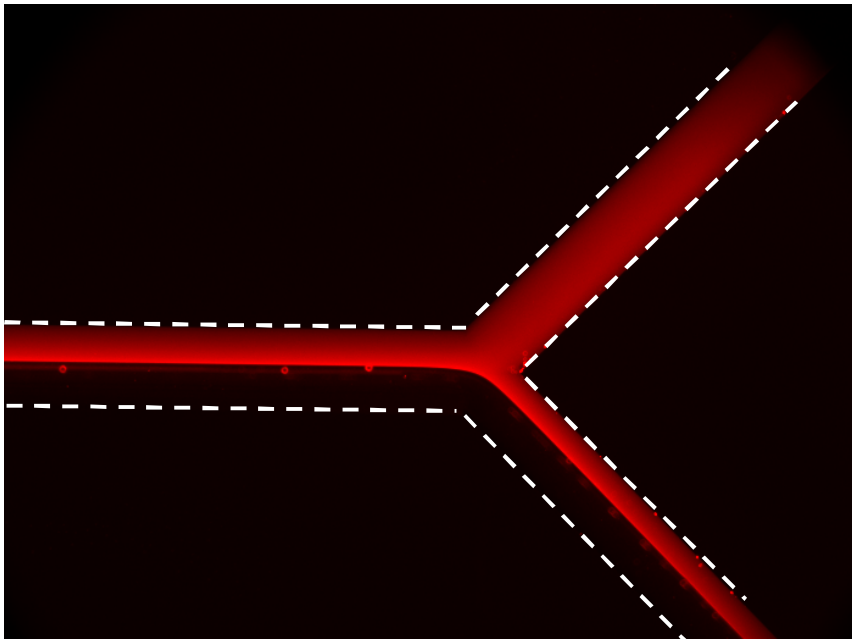
Splitting flow at outlet

- Apply a differential pressure to one outlet for precise shifting of interface
- Allows collection of desired phase with minimal contamination
- Maximal recovery of desired component requires:
  - Spreading of desired component away from interface
  - Precise split between phases, minimizing loss of desired phase

Pressure ↑



Pressure ↓



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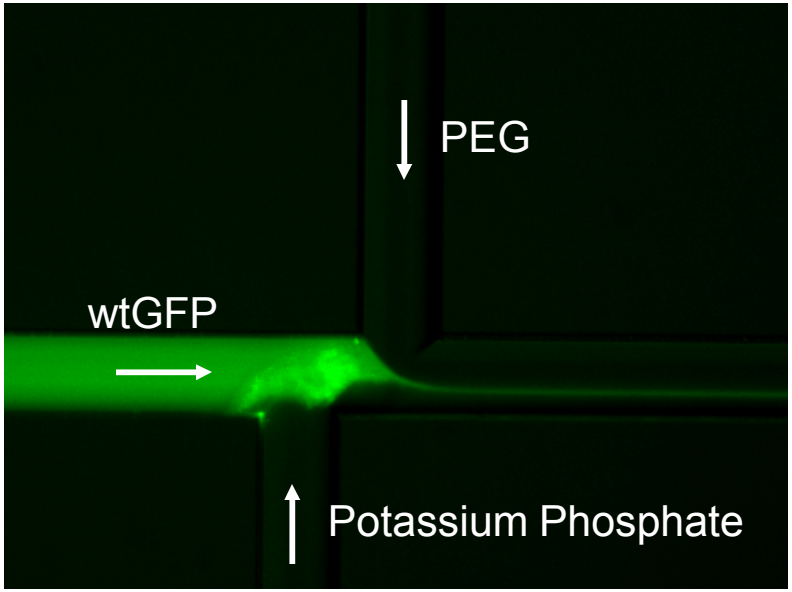
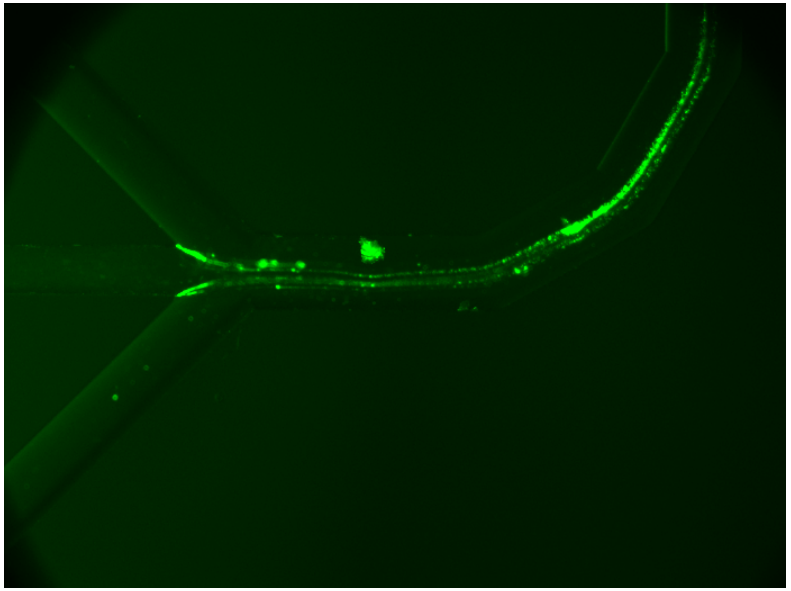
- Not all proteins are compatible with the high PEG and/or salt content
- Proteins may tolerate <15% or >50% PEG
- Addition of 0.01-0.1% Tween 20 can reduce aggregation and precipitation, although detergents can affect the phase equilibrium.



GFP precipitates with high salt concentration (Tween 20 helps prevent this)



Concavalin A precipitates badly in both PEG and salt even with Tween

[1]Albertsson, *Partition of Cells & Macromolecules*, 3rd ed, 1986

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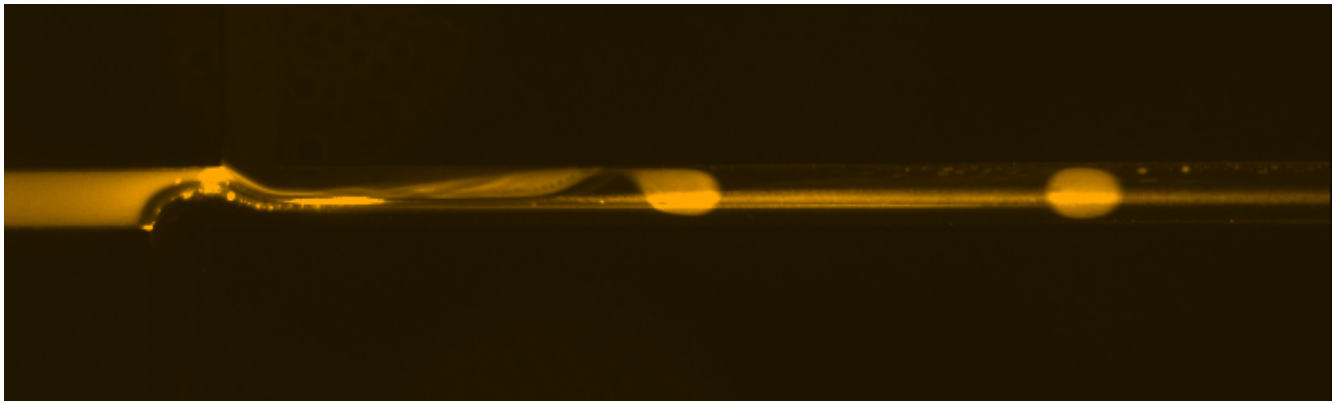
Segmented flow

- Droplets or segmented flow can occur
- Rapid equilibrium between two phases – long channels not required
- Interesting phenomenon, but separation of phases at outlet is difficult with a static, continuous flow device



Segmented flow with β-gal at low flow rate (~.4 psi ΔP, <1 mm/s) following precipitation at intersection

Future directions

- Optimize design of chip
  - Increase interfacial area per volume for faster mass transfer
  - Narrower channels will allow faster equilibration of concentration across channel while increasing residence time for a given ΔP and channel length
- Recovery of specific proteins from cell-lysate and assay for activity
- Integration of cell lysis with extraction on a single chip
- Explore approaches for further downstream processing (desalting, concentration, analysis)

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Amy Herr

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