Developing Peptide Concentration Gradients in a Membrane Environment

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Structure

- Introduction- Importance of cell binding to the ECM

- Motivation

- Purpose of Concentration Gradients

- Objectives

- Approach

- Microfluidic Devices and Microscopy Images

- Results

- Future Research

Introduction

- **Cells in multicellular organisms are anchored to extracellular matrix**

- **Intengrins and the RGD binding site**

- **Design of biomimetic materials depends on understanding cell behavior on functionalized surfaces**
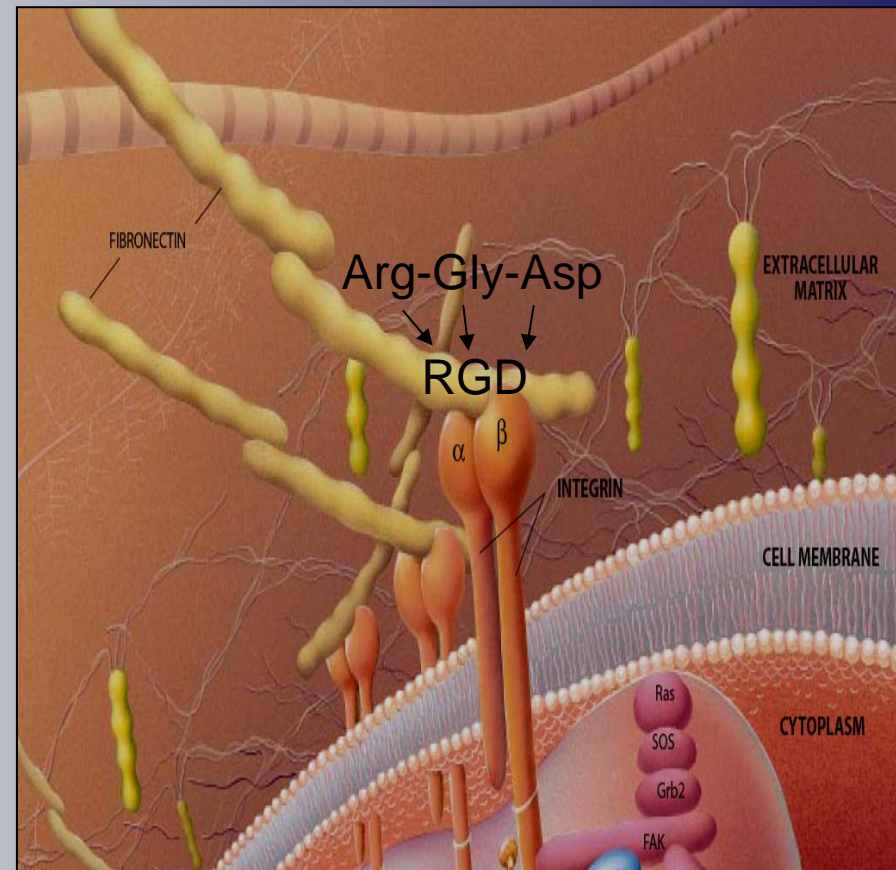
"Integrins and Health." Horwitz Alan F. Scientific American May 1997
http://www.med.unc.edu/~meissner/sciamer.pdf

What is a Concentration Gradient?

- **Change in concentration of a solute across a distance**
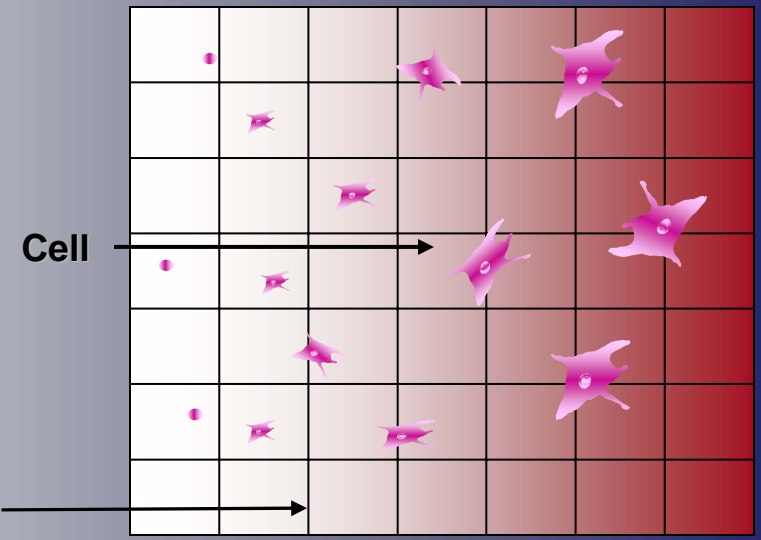- Many different concentrations on a single surface

Why Research Peptide Concentration Gradients?

- Contribute knowledge for the development of surface properties that promote favorable reactions by cells

- Future contribution to bio-engineered materials
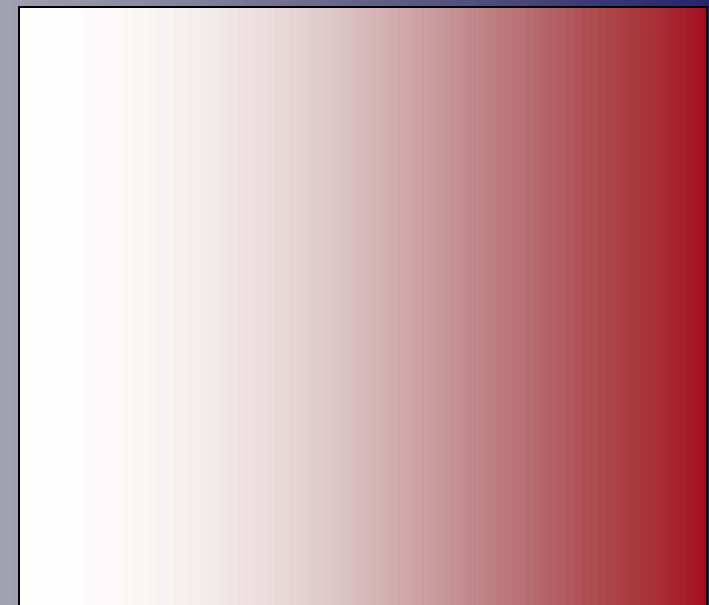
Solution less concentrated
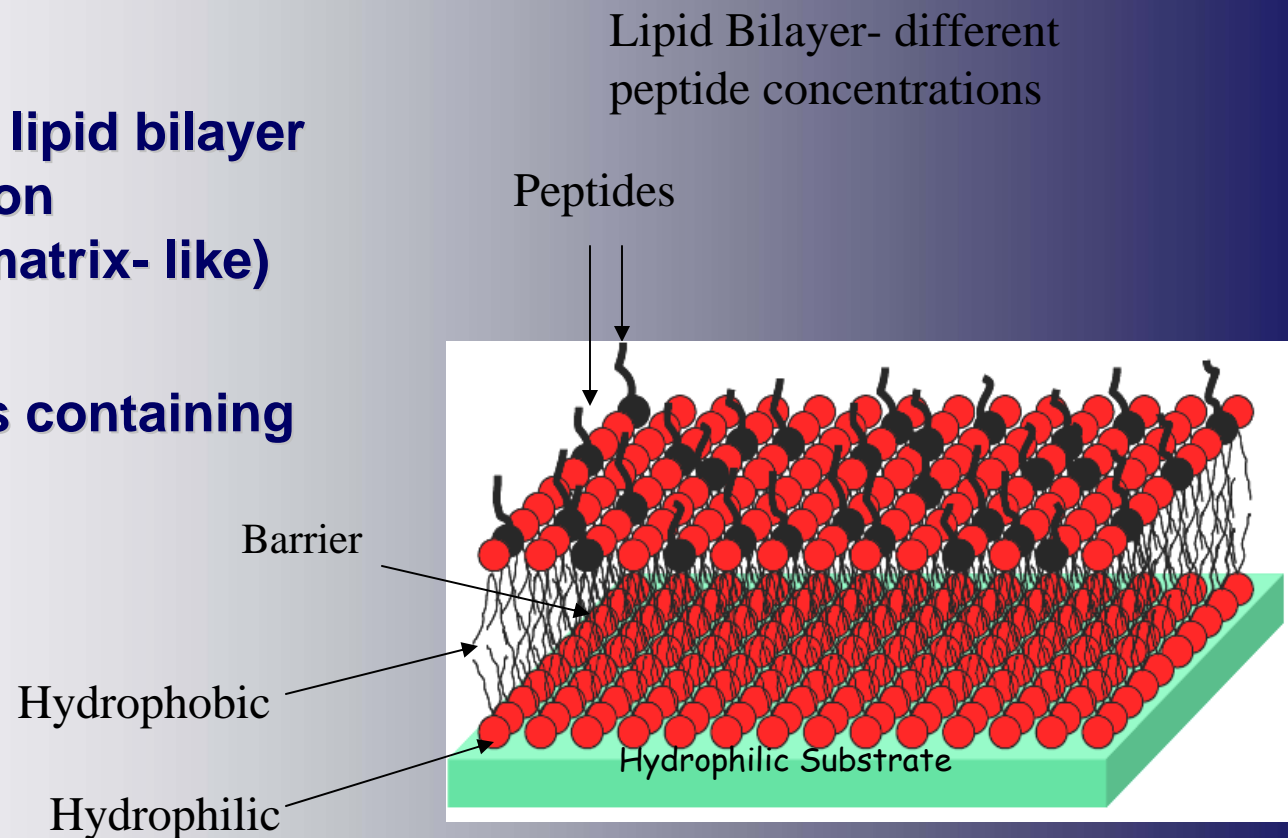
Solution more Concentrated

Cell

Diffusion Barriers

Picture courtesy by Dimitris Stroumpoulis

Objectives

- **Create surface concentration gradients using vesicle solutions**

- **Formation of a lipid bilayer by vesicle fusion (extracellular matrix- like)**

- **Use of vesicles containing RGD peptides**

Lipid Bilayer- different peptide concentrations

Peptides

Barrier

Hydrophobic
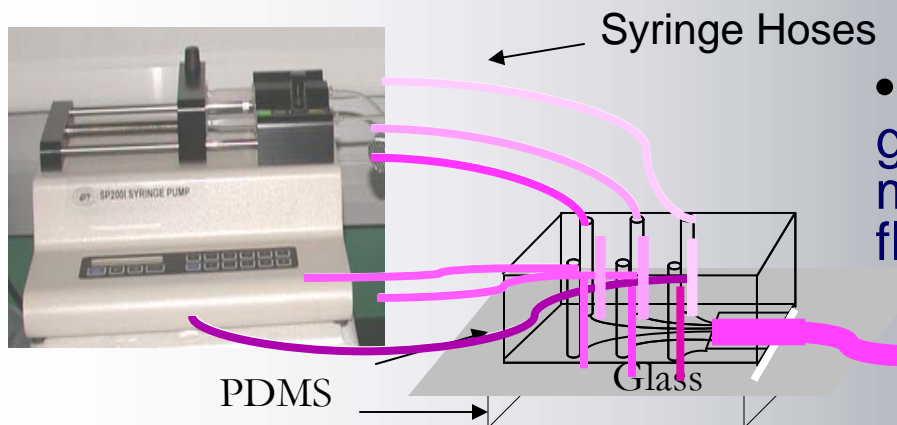
Hydrophilic

Hydrophilic Substrate

Picture Courtesy by Dimitris Stroumpoulis

Experimental Methods

- Poly(dimethylsiloxane) (PDMS) mold from wafer

    -Create inlets and outlet

    -Glass slide treated with plasma $\longrightarrow$ hydrophilic
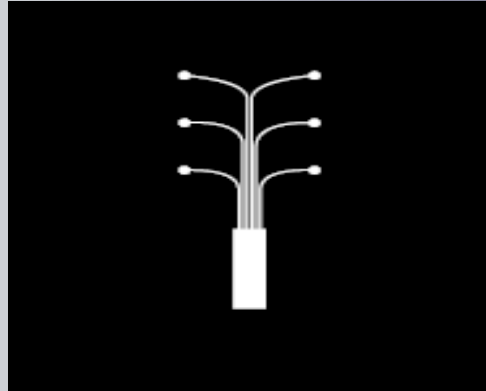
- Flow of vesicle solutions
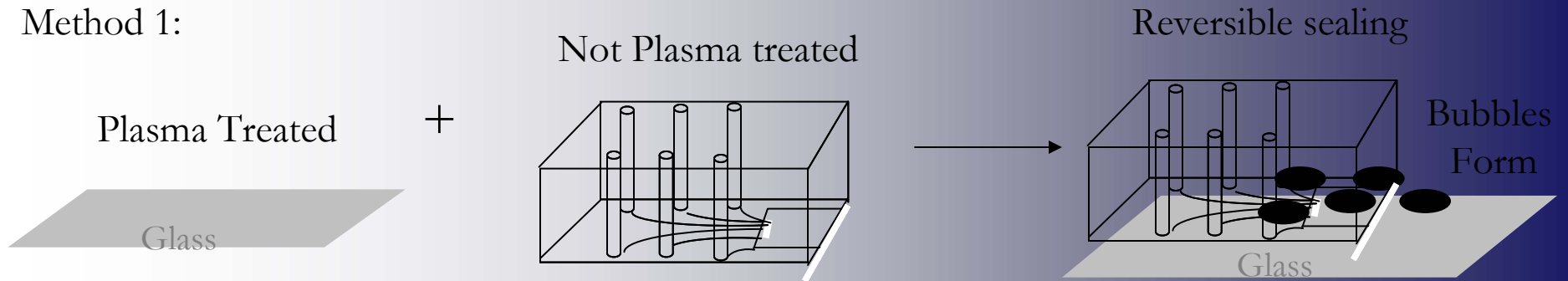   -Egg (PC) (lipids) & Texas Red (lipids and fluorescent dye)

    -No peptides

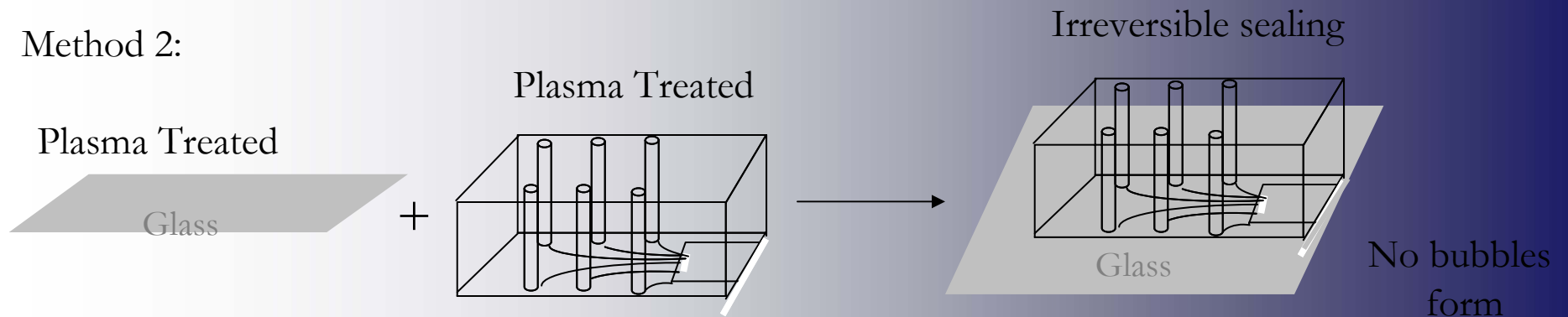- Observation of gradient using microscope and fluorescent light

Syringe Hoses

PDMS

Glass

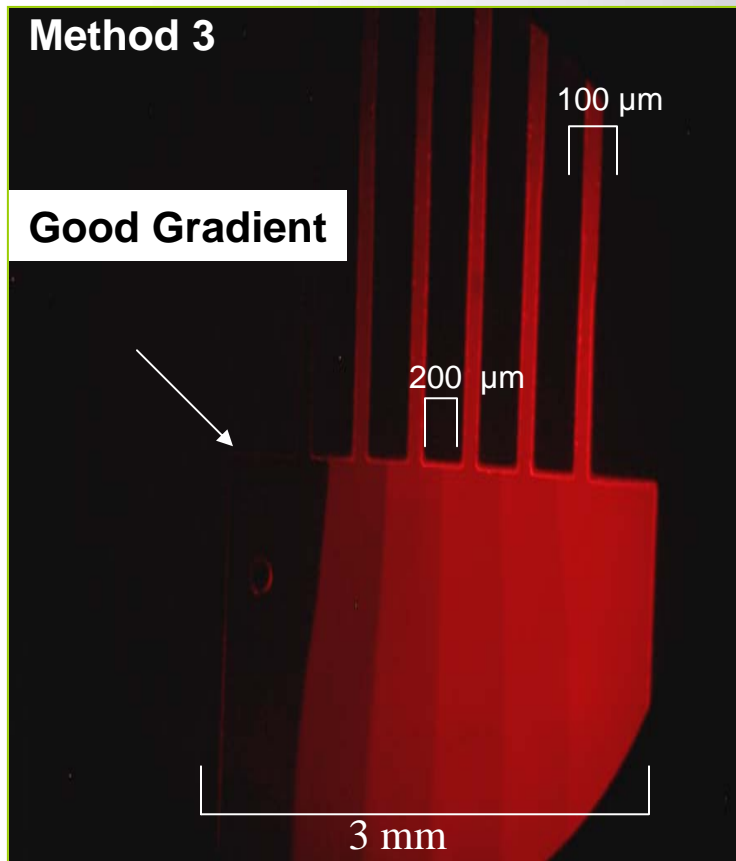# Microfluidic Devices

Method 1:

Plasma Treated

Glass

$+$

Not Plasma treated

Reversible sealing

Bubbles Form

Glass

Method 2:

Plasma Treated

Glass

$+$

Plasma Treated

Irreversible sealing

Glass

No bubbles form

Method 3:

Glass

Bonded and Plasma Treated
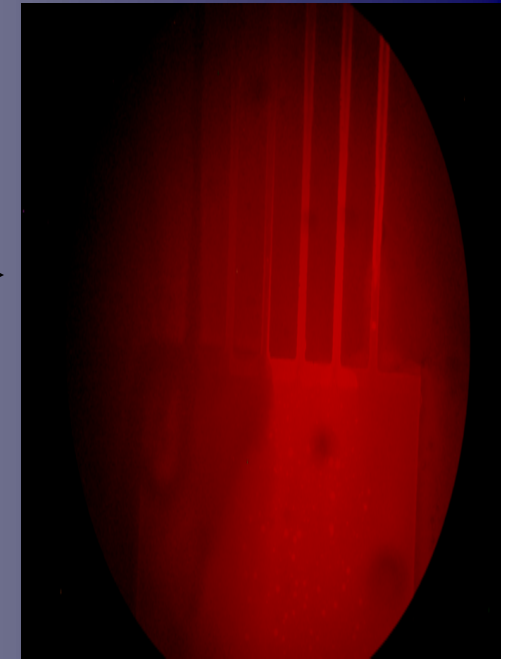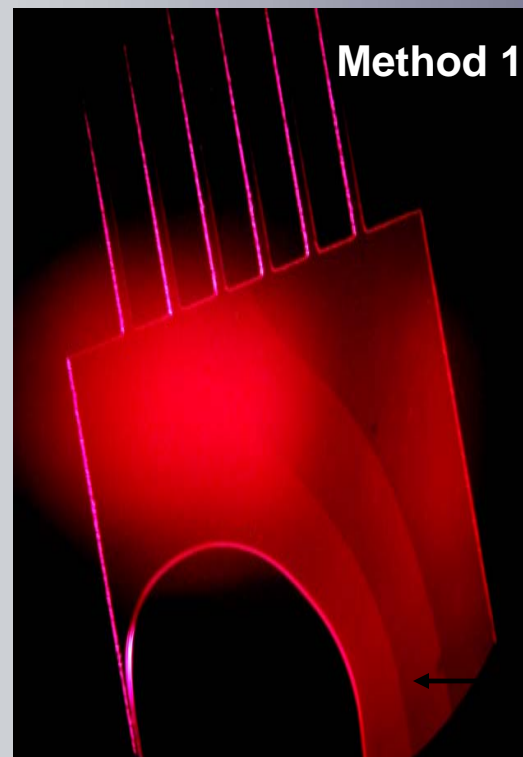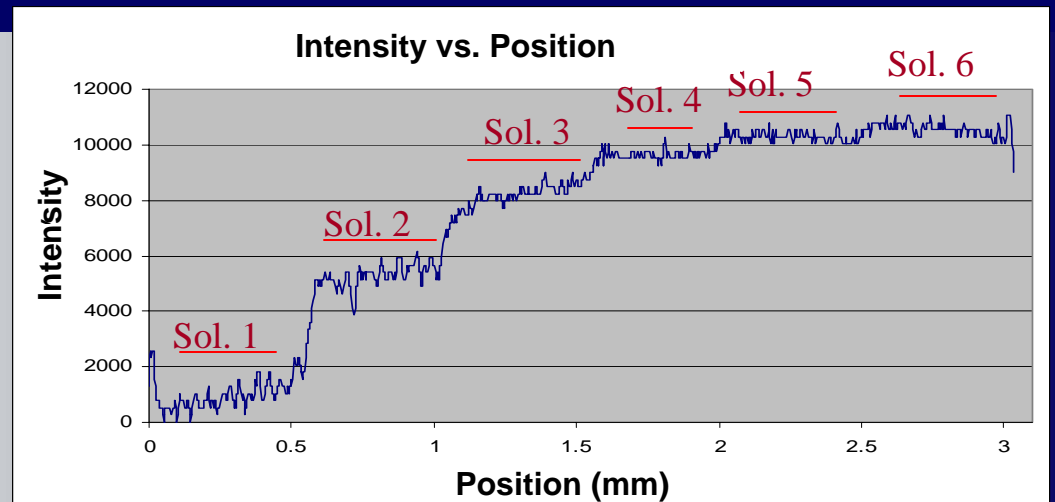
Reversible sealing & No bubbles form

Glass
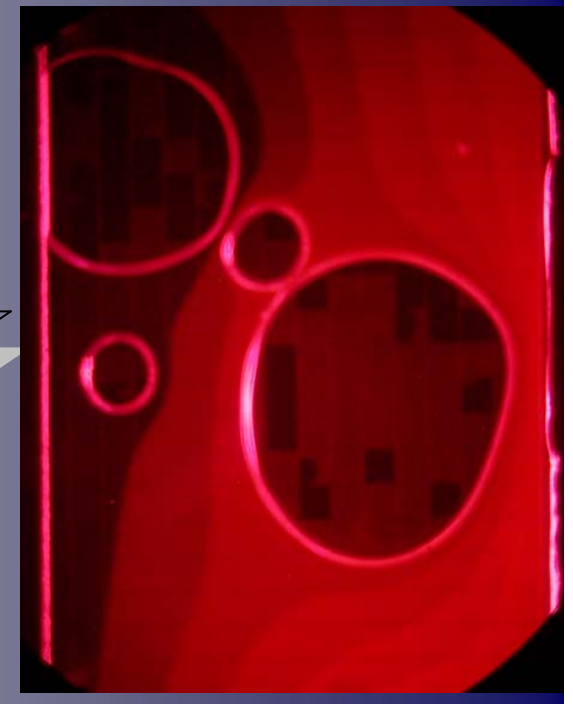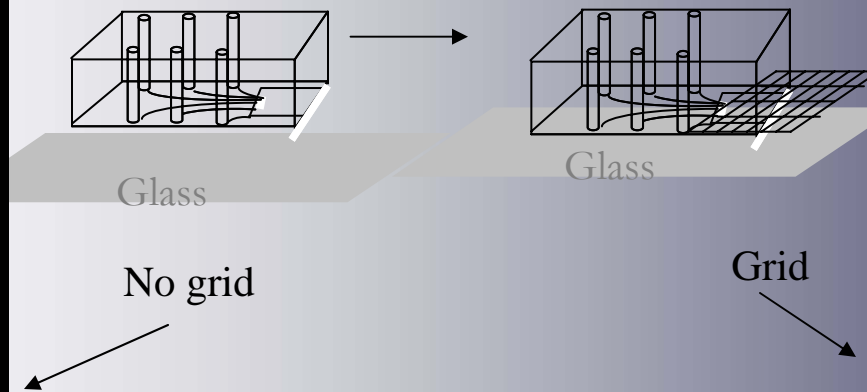
Microscopy Images

- Gradients viewed under fluorescent light

Method 3

Good Gradient

100 µm

200 µm

3 mm

Poor Gradient

Method 1

Bubble

Results

Solution: 1 2 3 4 5 6

Intensity vs. Position

Sol. 1, Sol. 2, Sol. 3, Sol. 4, Sol. 5, Sol. 6
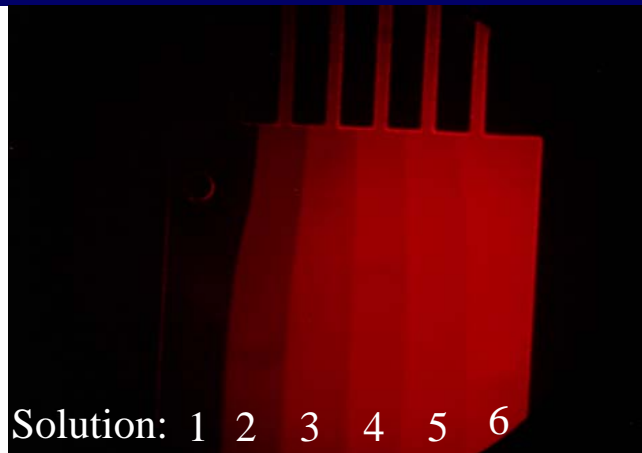
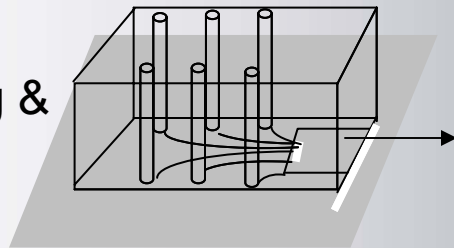- From glass slide to glass containing grid

Glass

No grid

Glass

Grid
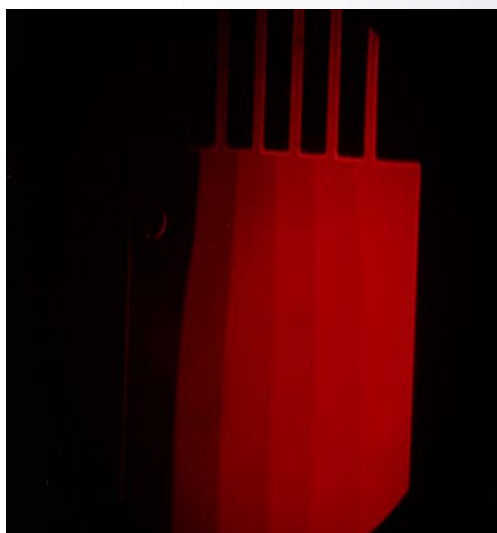
Summary and Future Research

Summary
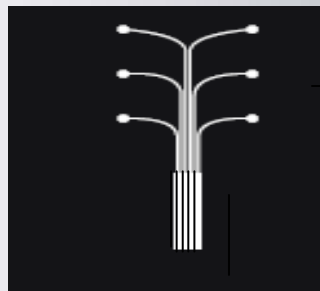
Reversible sealing & no bubbles

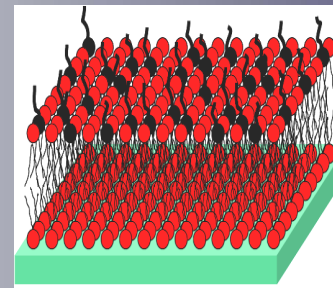Creation of gradient in solution

Use of new microfluidic channel design

Creation of gradient on glass containing barrier

Use of vesicles containing Peptides

Observation of bilayer under Microscope

Future Research

- Research cell behavior (e.g. cell migration & cell spreading) on peptide surface bound concentration gradient surfaces
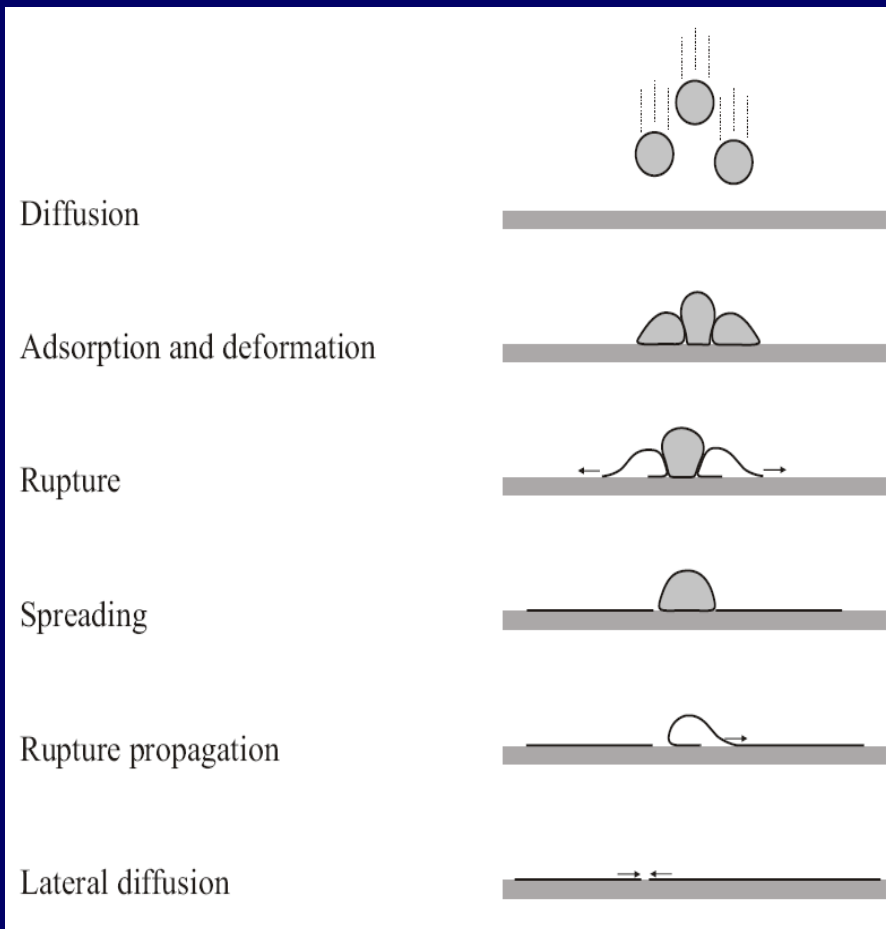
Acknowledgements

I would like to thank…

❖My Mentor Dimitris Stroumpoulis

❖Matthew Tirrell- Faculty Advisor

❖INSET Program

Solution Concentrations

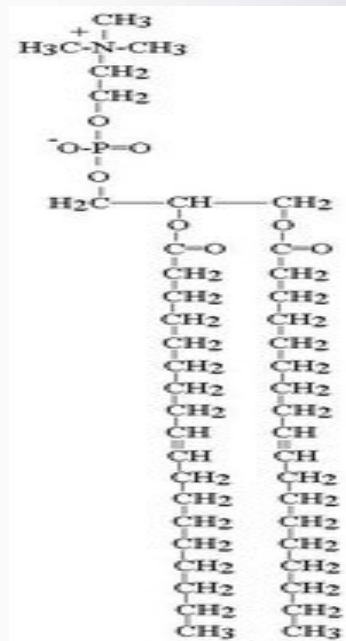|	Egg PC	Texas Red	Buffer	Total
Solution 1	1.5 ml	0 ml	1.5 ml	3 ml
Solution 2	1.2 ml	0.3 ml	1.5 ml	3 ml
Solution 3	0.9 ml	0.6 ml	1.5 ml	3 ml
Solution 4	0.6 ml	0.9 ml	1.5 ml	3 ml
Solution 5	0.3 ml	1.2 ml	1.5 ml	3 ml
Solution 6	0 ml	1.5 ml	1.5 ml	3 ml

Vesicle Fusion

Diffusion

Adsorption and deformation

Rupture

Spreading

Rupture propagation

Lateral diffusion

Picture Courtesy by Dimitris Stroumpoulis

Lipids Used

- Egg phosphatidylcholine (PC)

•http://en.wikipedia.org/wiki/Phosphatidylch
oline