Dissolution Modeling of Uniform Aqueous Droplets in Two-Phase Flow in a Microfluidic Device

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Outline

- Background and Motivation
- Biopreservation
- Novel microfluidic thermal system
- Mathematical modeling and simulation

Background

CPA : Cryo-protectant agent

Carbohydrate based CPAs are added to accommodate for ultrafast cooling rates required for vitrification

CPAs prevent intra- /extra-cellular crystallization by increasing the glass transition temperature at the cost of toxicity.

Novel approaches:

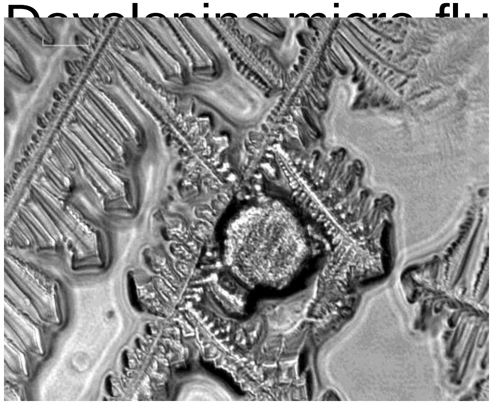
Develop novel technologies for safer cryopreservation by

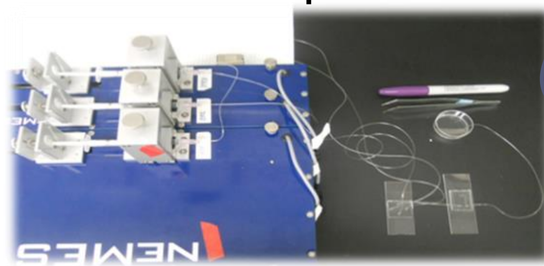
- modifying the protocols
- controlling the loading and concentration of CPAs in the cell prior to freezing.

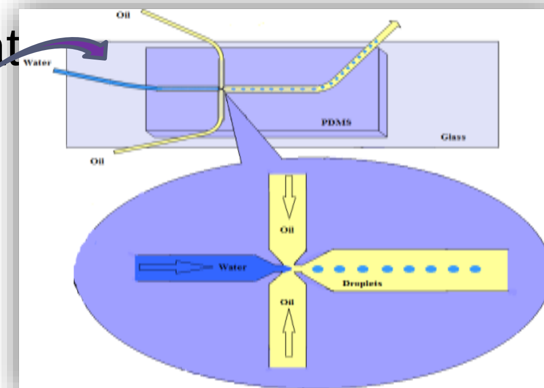
Micro Channel Thermo-Fluidic Device

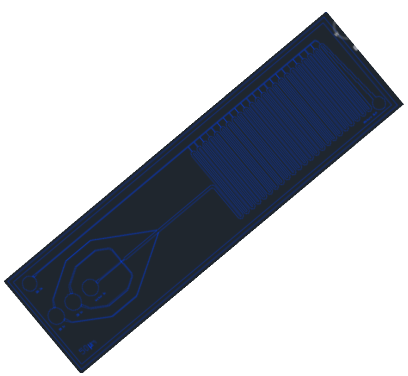
Harvard Medical School + MIT + MGH → Center for Engineering in Medicine, Boston

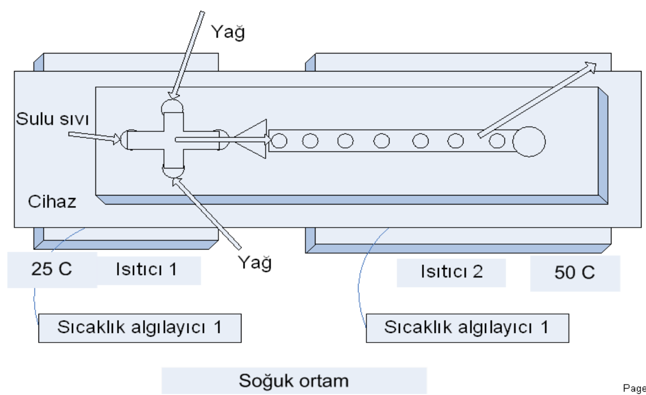
- Prevention of crystallization in biopreservation
- Developing micro-fluidic device for preconcentrat

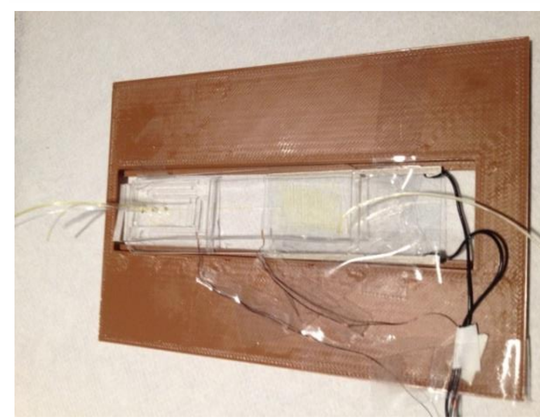






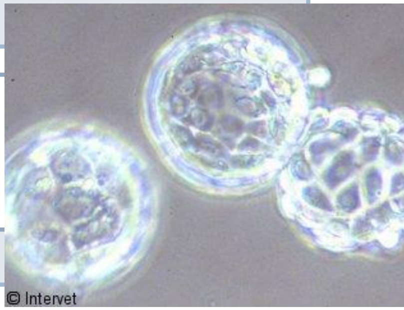






Biopreservation Methods

- In-vitro culture
- Hypothermic storage



- Cryopreservation
- Dry-preservation (desiccation)

In vitro culture

Replication of natural environment ex vivo

↓

Storage at physiological T

- Tight control of physiological/physicochemical determinants
- Short term / Small amounts
- Continuous quality control and media replacement
- Costly

Vaccines, Monoclonal antibodies, Recombinant proteins, Cytokines, Other therapeutic agents

Hypothermic storage

Goal: Suppress molecular/biochemical reactions

$T_{freezing,\ solution} < T_{storage} < T_{physiological}$

Advantages
- Inexpensive
- Commercially available hypothermic preservation solutions

Disadvantages
- Slowed cellular metabolism (not suppressed)
- Cell damage
- Short term

Dry preservation

Addition of agents less active than water

Trehalose, glycerol, dextran...

↓

Removal of water from cell

↓

Vitrification (glassification)

↓

Long term storage @ T_{room}

inexpensive

Cryopreservation

@ ultralow temperatures (<-150°C)

↓

Freeze biological structure/function of living systems

✓ Relatively longer storage time

☒ Special equipment

☒ Specialized personnel

☒ High concentration of cryoprotectant agents (CPA)

☒ Cell damage during freezing

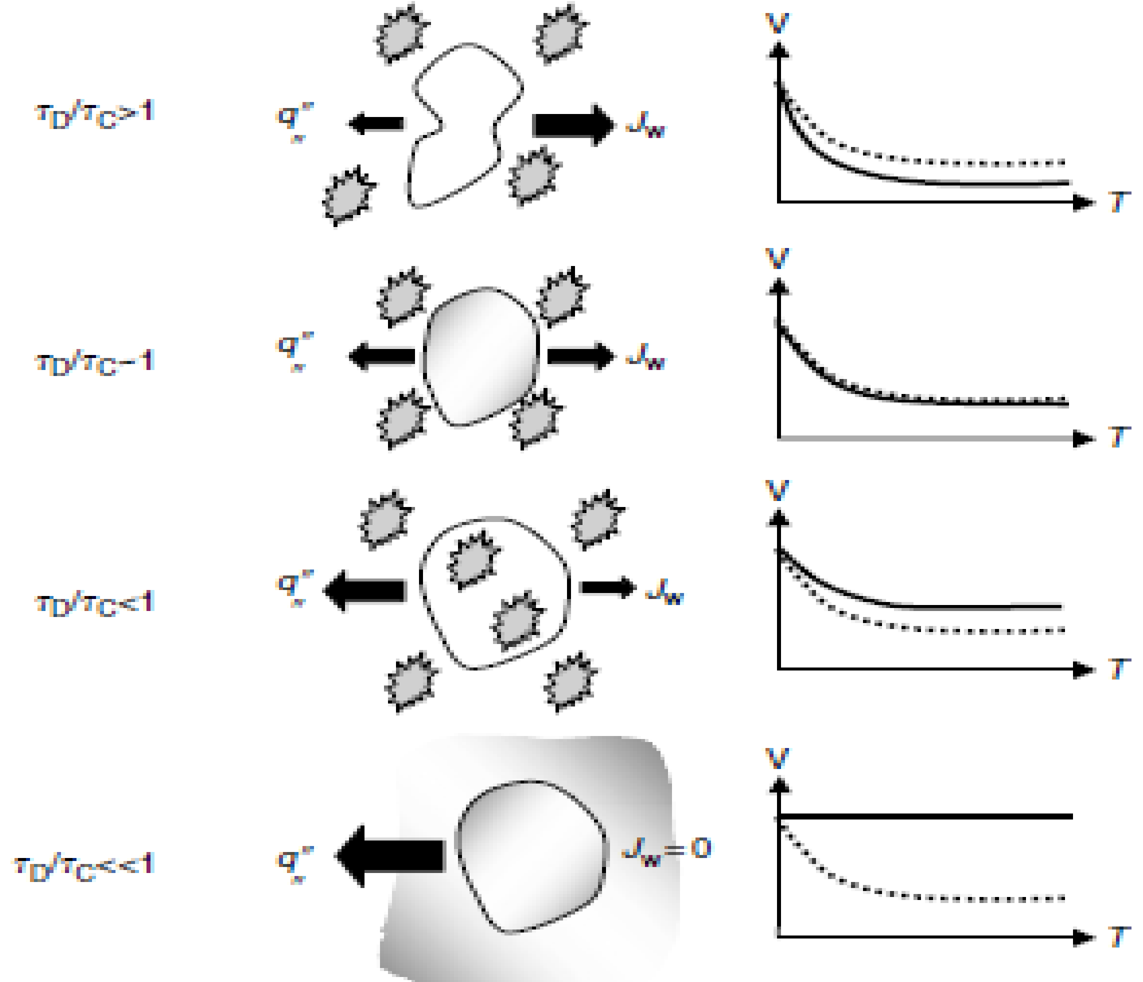
Crystallization

Crystallization →Harmful

Vitrification →Safe

Water → Glass

1. Cooling rate ~1000000 C/s
2. CPA↗ Cooling rate↘

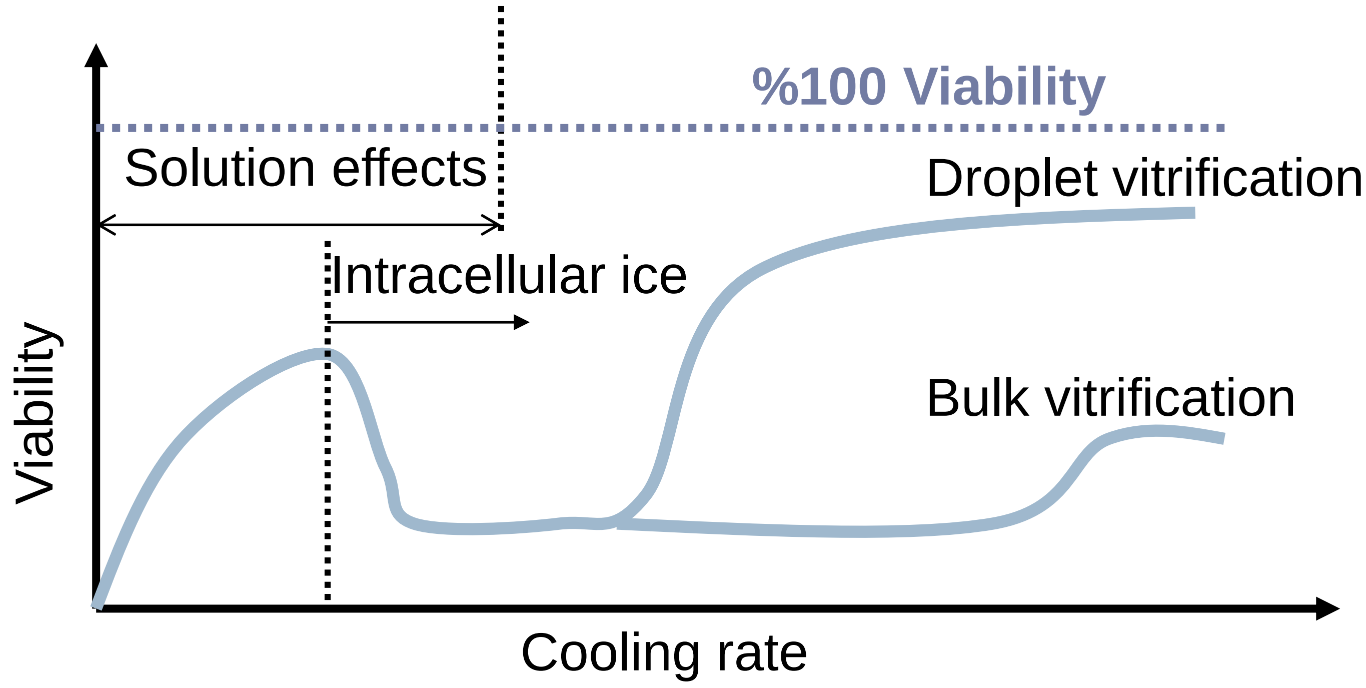


$$\tau_D = \frac{r}{3L_p\Delta\Pi}$$

$$\tau_C = \frac{2c_p\rho r\Delta T}{3q''}$$

Reference: Aksan A ve Toner M, 2

Cooling rate

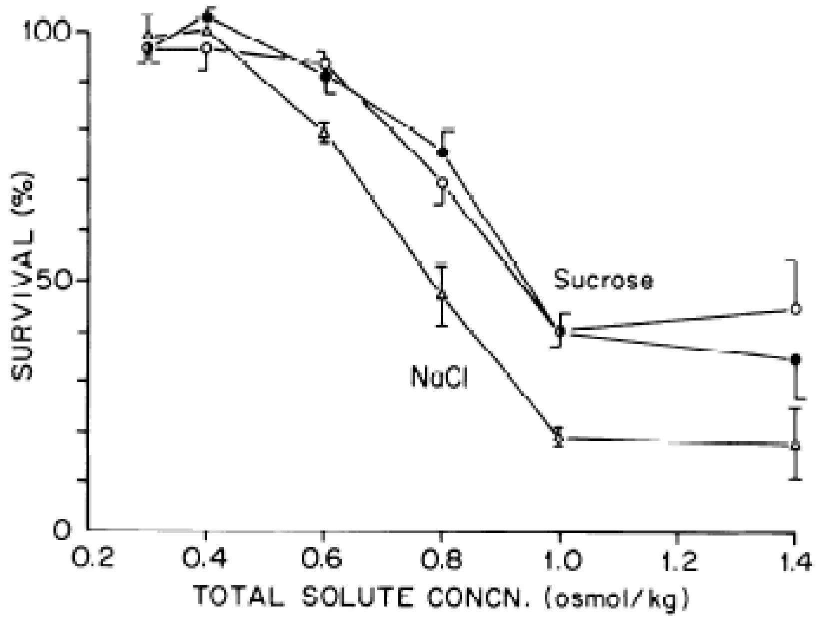


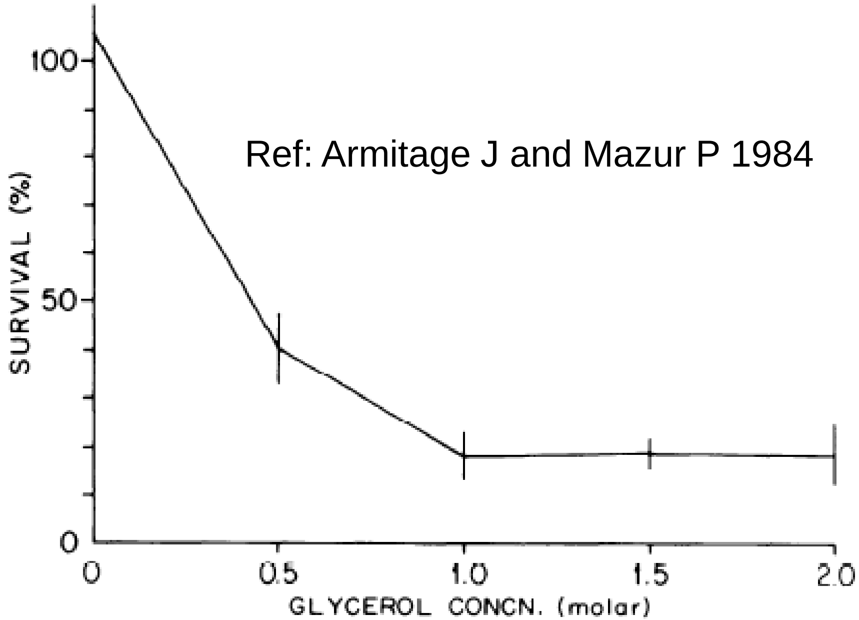
CPA effect

Human granulocytes

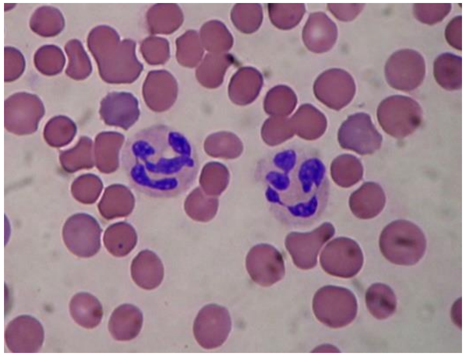
2M glycerol

>5 min → 50% viability





Ref: Armitage J and Mazur P 1984



Engineering solutions

- Speeding up heat transfer and cooling → Down-sizing
- Reducing CPA concentration
- **Reducing CPA exposure time**

  → Controlled CPA loading

Goal

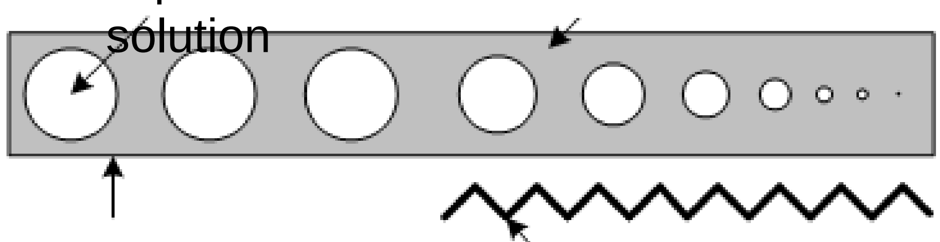
Develop a novel micro-channel thermo-fluidic device to control and improve the rate of dehydration in biomaterials

1. Produce uniform picoliter aqueous droplets in a mobile organic phase in continuous flow
2. Optimise operating conditions of the device to establish a steady-state two-phase laminar flow following droplet formation
3. Control the system thermally to allow for interphase diffusion of water
4. Encapsulate one or more cells per droplet

Microfluidic solution

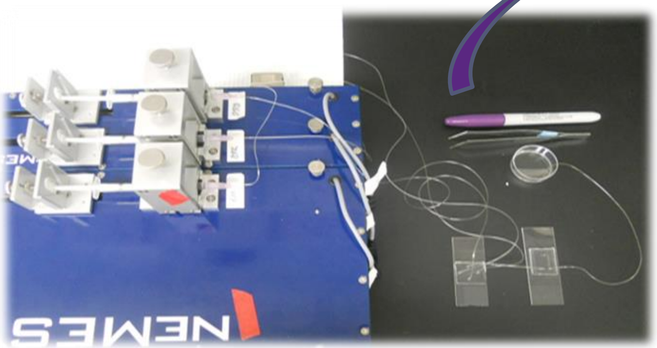
- Microchannel device made of poly-dimethyl-siloksane (PDMS)
- Hydrophobic surface
- Organic phase: Soybean oil– triacylglycerol

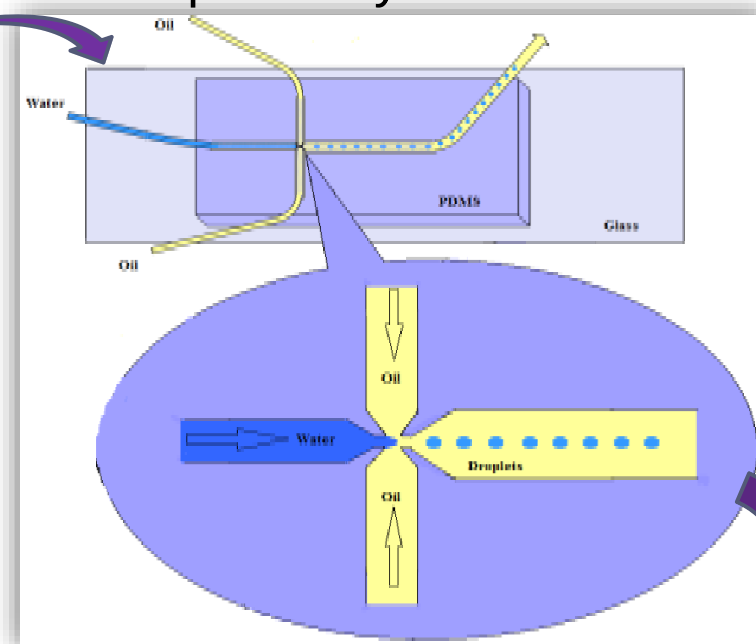
  water solubility f(T) 0.3%

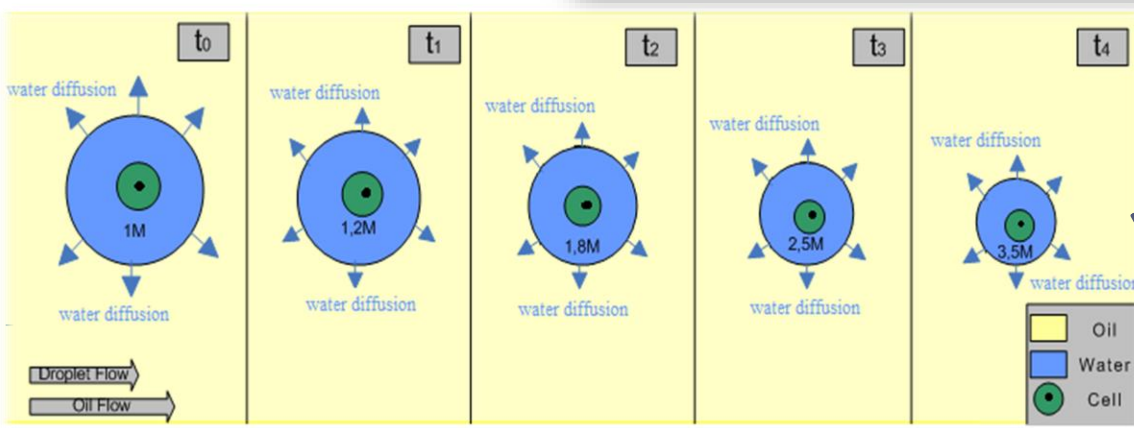


Mechanism

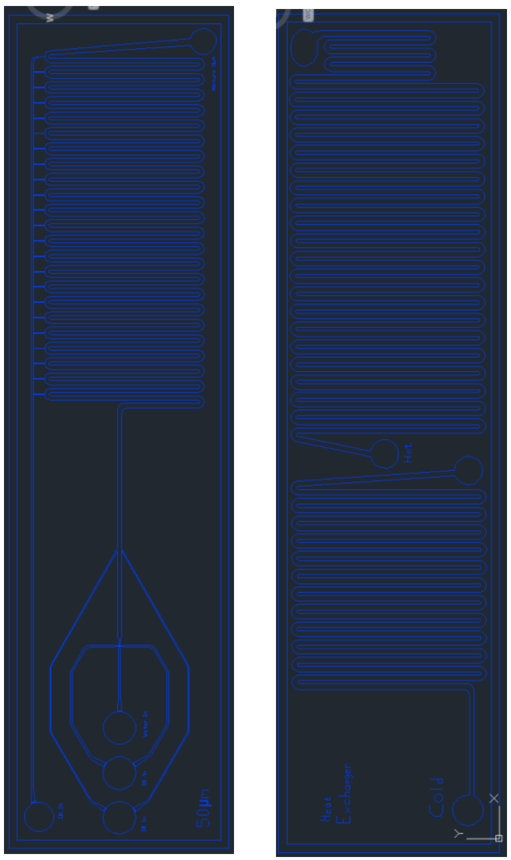
- Inverted fluorescence microscope and camera
- Syringe pump system with micro-flow control
- ITO heater, micro T sensor, micro T control and power system



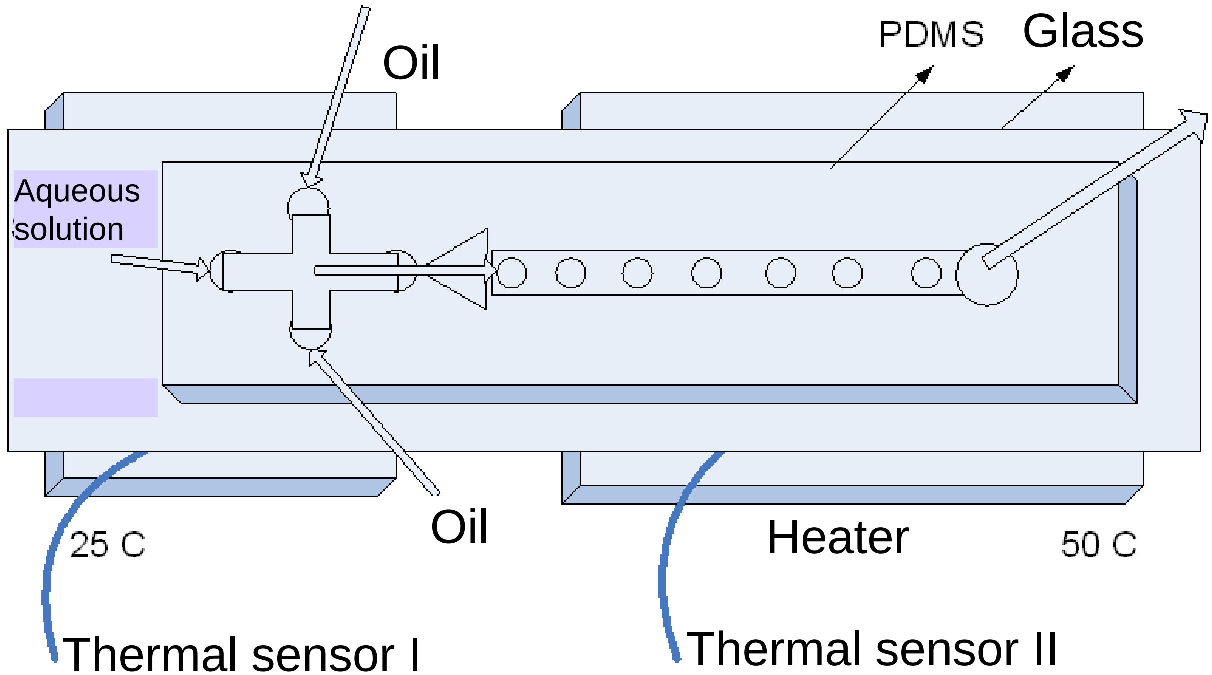


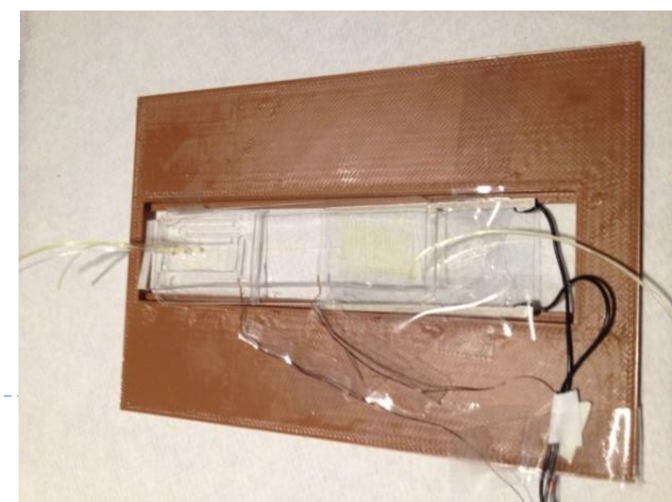


Micro-channel thermo-fluidic device

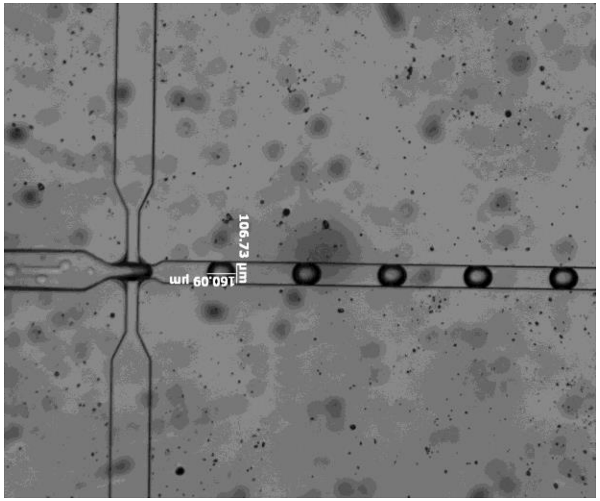


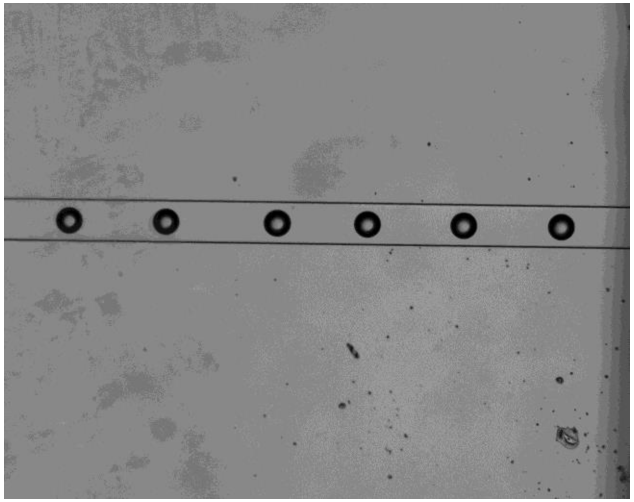
AutoCAD design

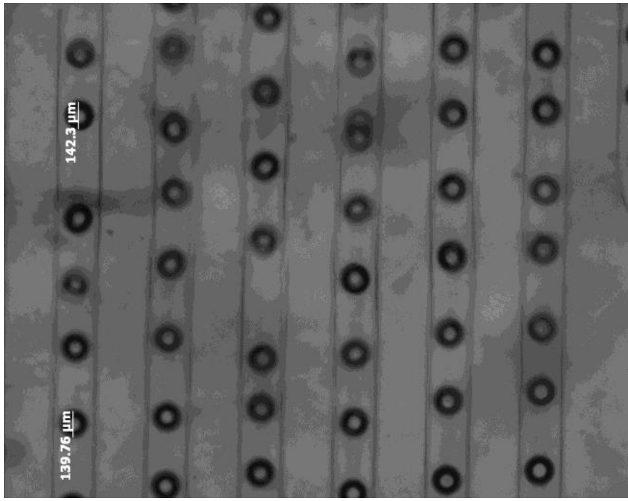




Pictures in phase

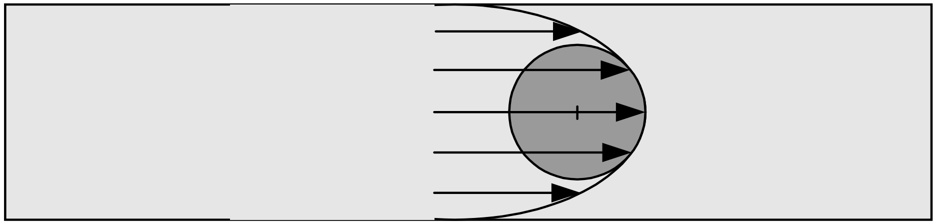






Mathematical Model

- COMSOL.Multiphysics
- MATLAB



Conservation of mass
Ficks 2nd law

$$J_i = -D \cdot \nabla C_i + u \cdot C_i$$

$$\frac{dC_i}{dt} = \nabla \cdot (D \cdot \nabla C_i) - u \cdot \nabla C_i$$

Laminar flow
Conservation of momentum
Navier-Stokes equations

$$\rho \nabla \cdot u_i = 0$$

$$\rho \cdot \frac{\delta u_i}{\delta t} + \rho (u_i \cdot \nabla) u_i = \nabla \cdot \left[-p_i + \mu (\nabla u_i + (\nabla u_i)^T) \right] + F_i$$

Flow in channel
Newton's 2nd law

$$U_i = \left(r_i^2 - \frac{w^2}{4} \right) \cdot \frac{\Delta P}{L \cdot \mu \cdot 2}$$

COMSOL Multiphysics® Tools

Mathematical model: Unsteady state

Two-phase flow & Mass transfer through a moving boundary layer

-Transport of Diluted Species interface: Calculation of mass transfer

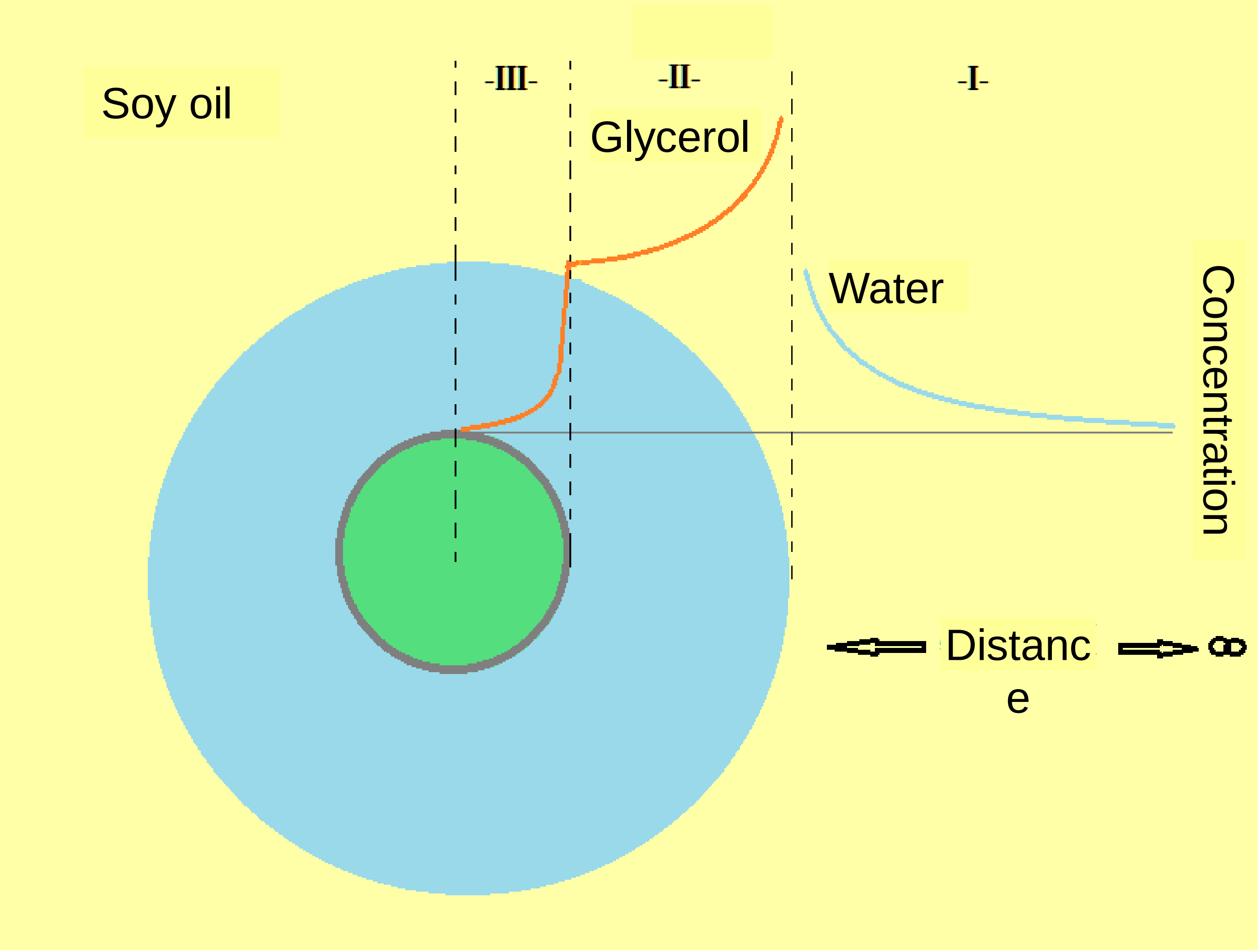
Thin Diffusion Barrier boundary condition used to create the effect of a cell membrane.

-Laminar Flow interface for the calculations of fluid flow

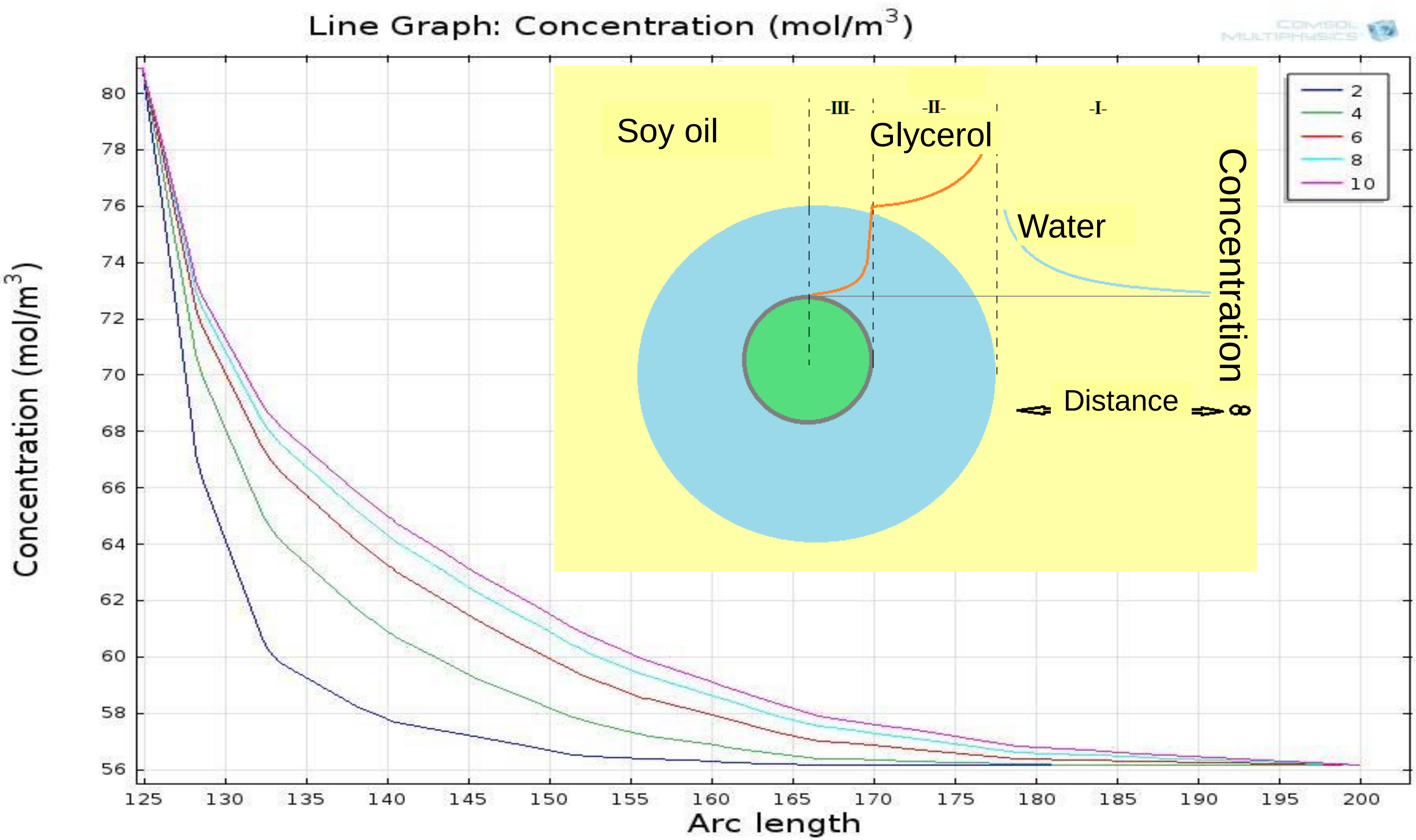
-Moving Mesh interface to create the moving boundary during shrinkage of aqueous droplets.

Curve-fits in MATLAB®: Fluid flow parameters (relative velocity) from the resulting data imported from the COMSOL® and exported back

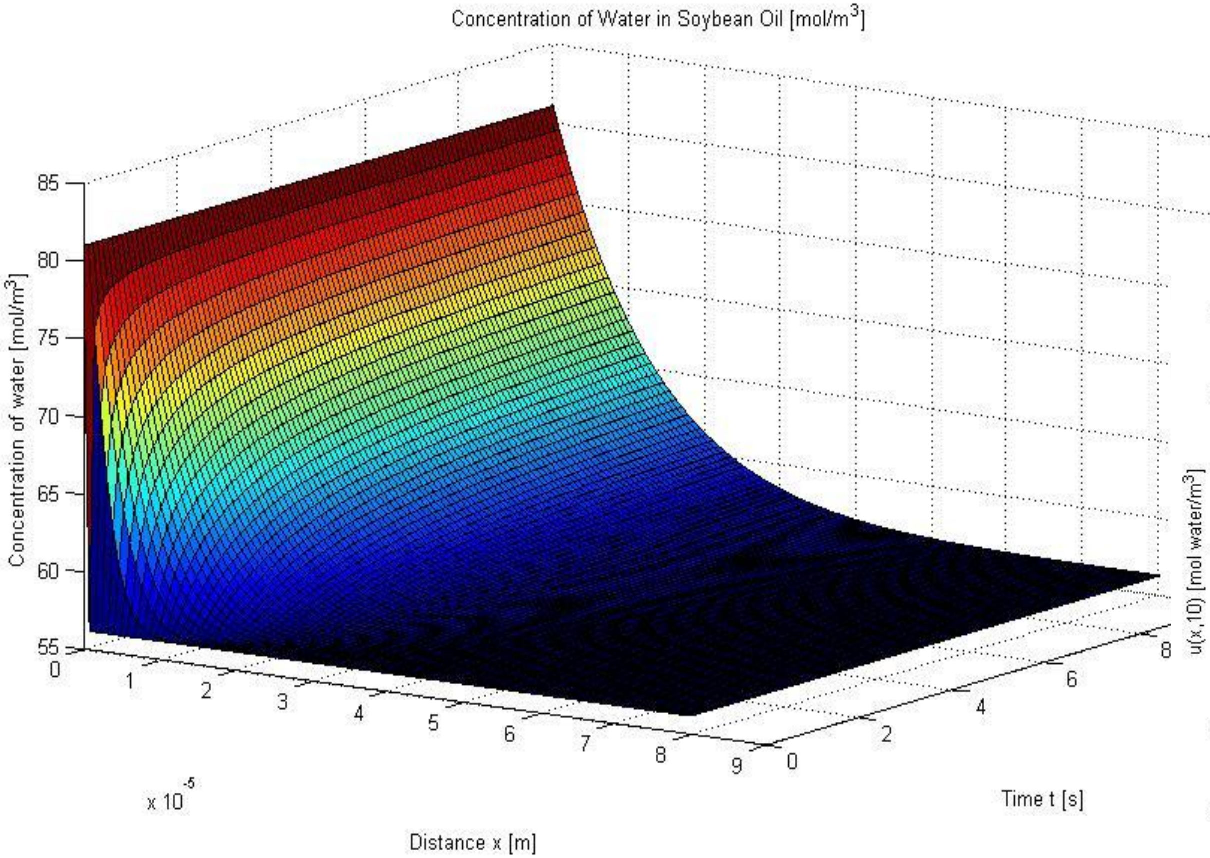
Intra/Extracellular water and glycerol concentration profiles

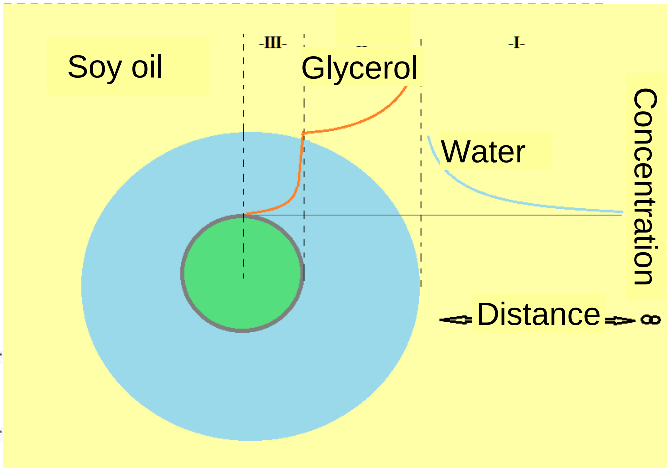


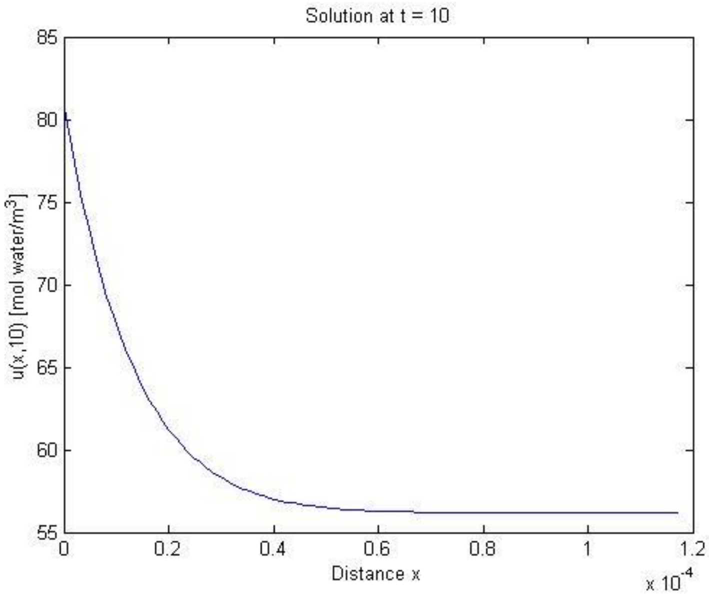
Aqueous concentration in oil - COMSOL



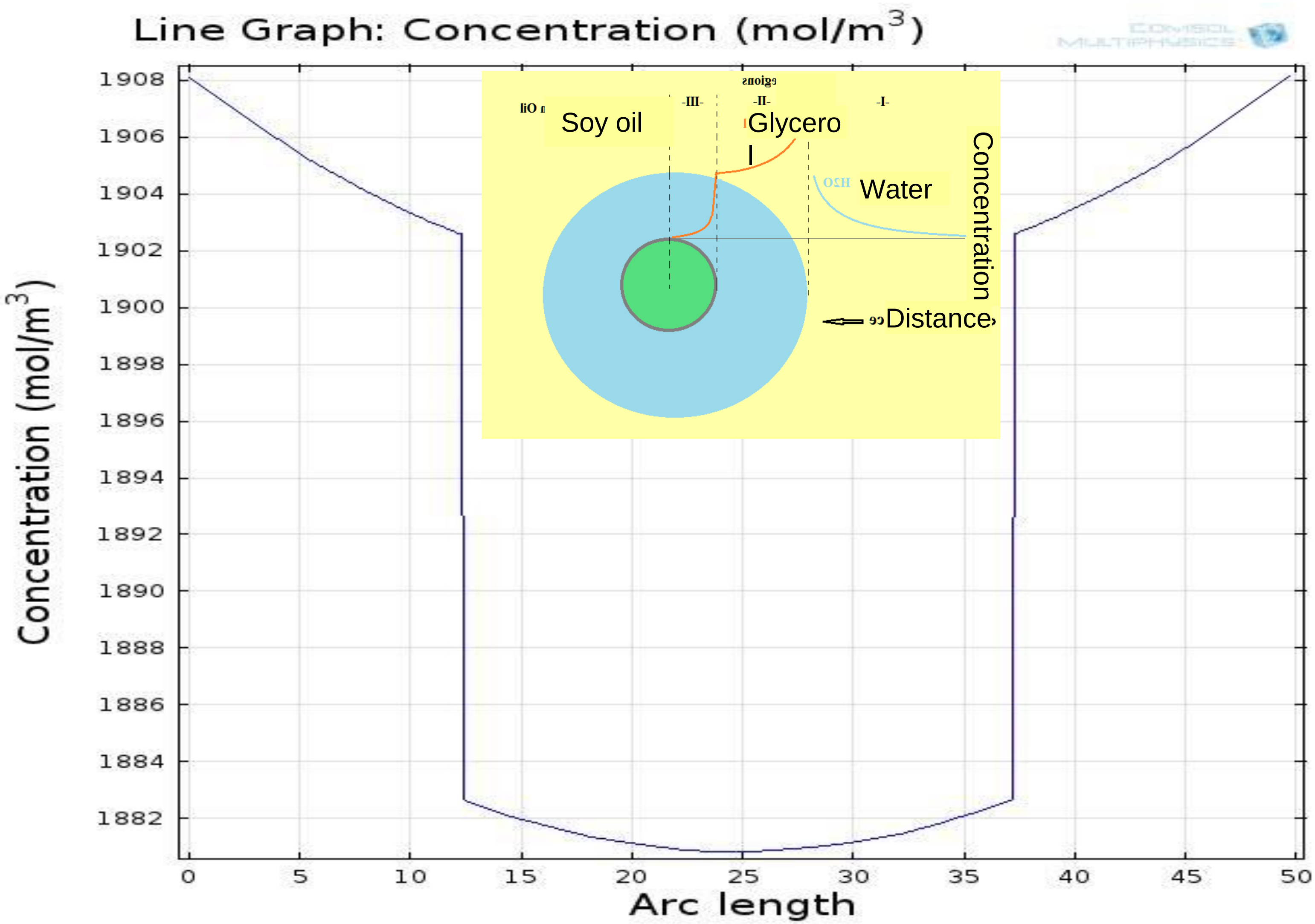
Aqueous concentration in oil - MATLAB



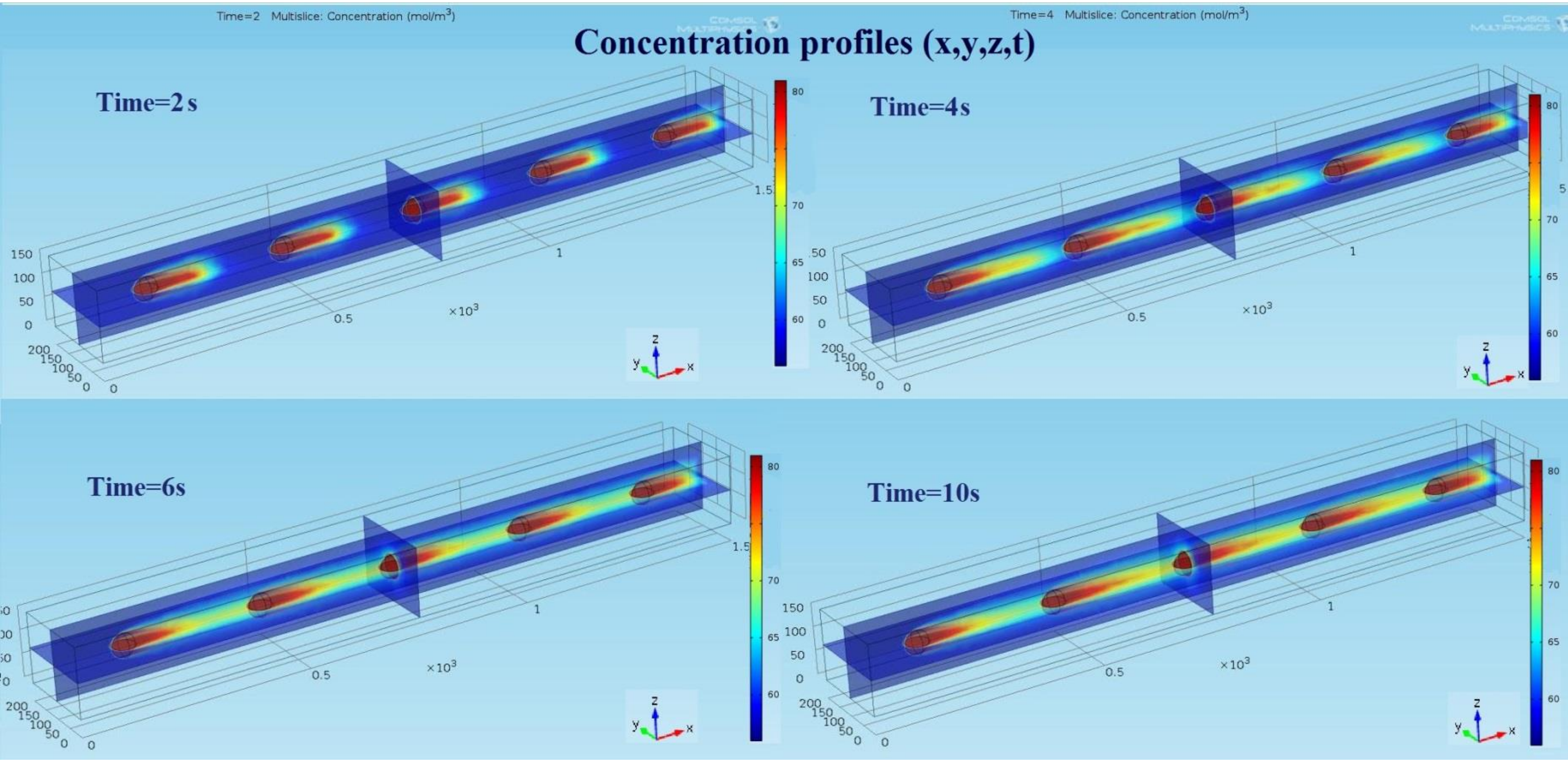




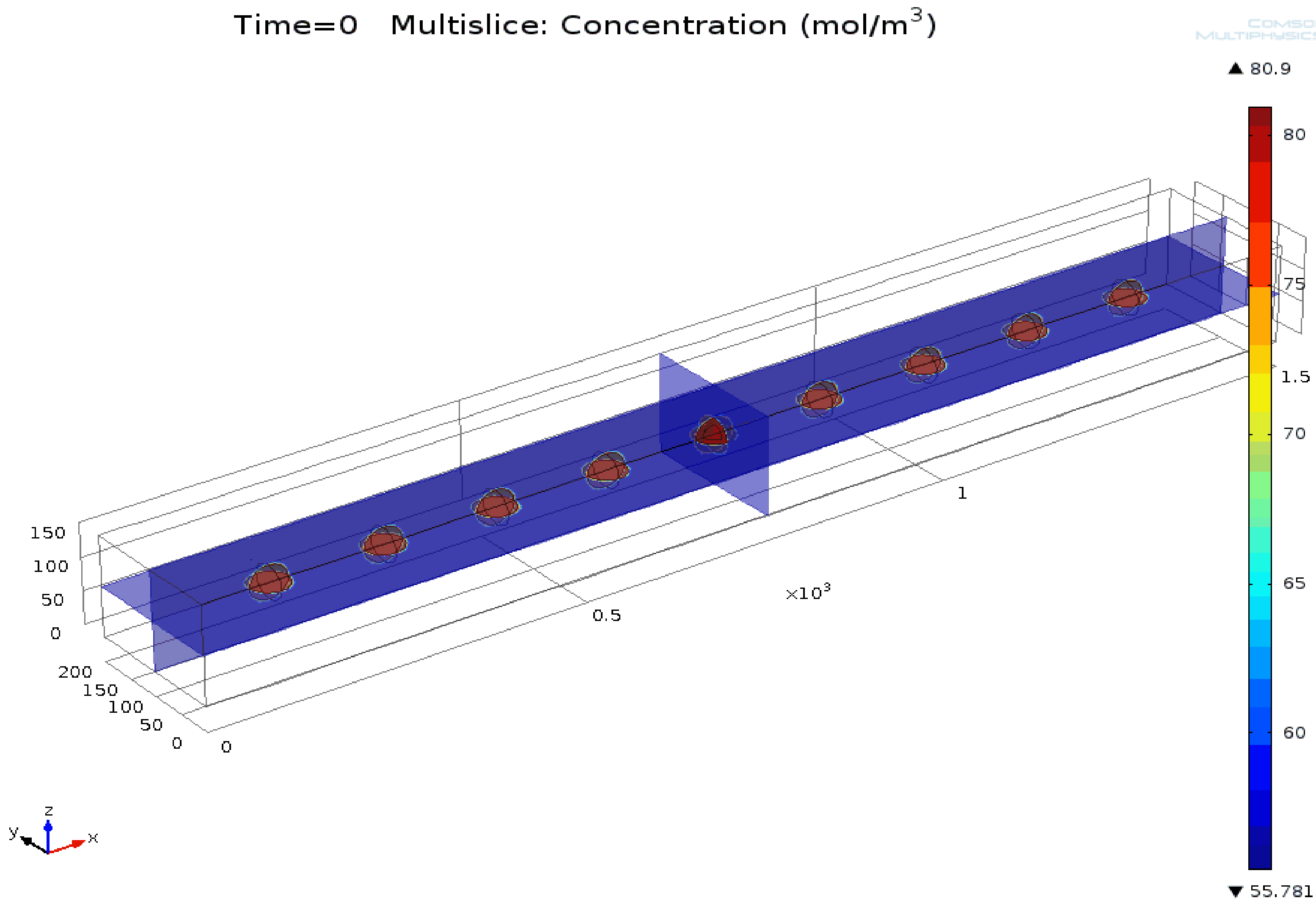
Intra-/Extra-cellular concentration of glycerol



Flow concentration profiles-COMSOL



In-channel aqueous concentration profile during flow



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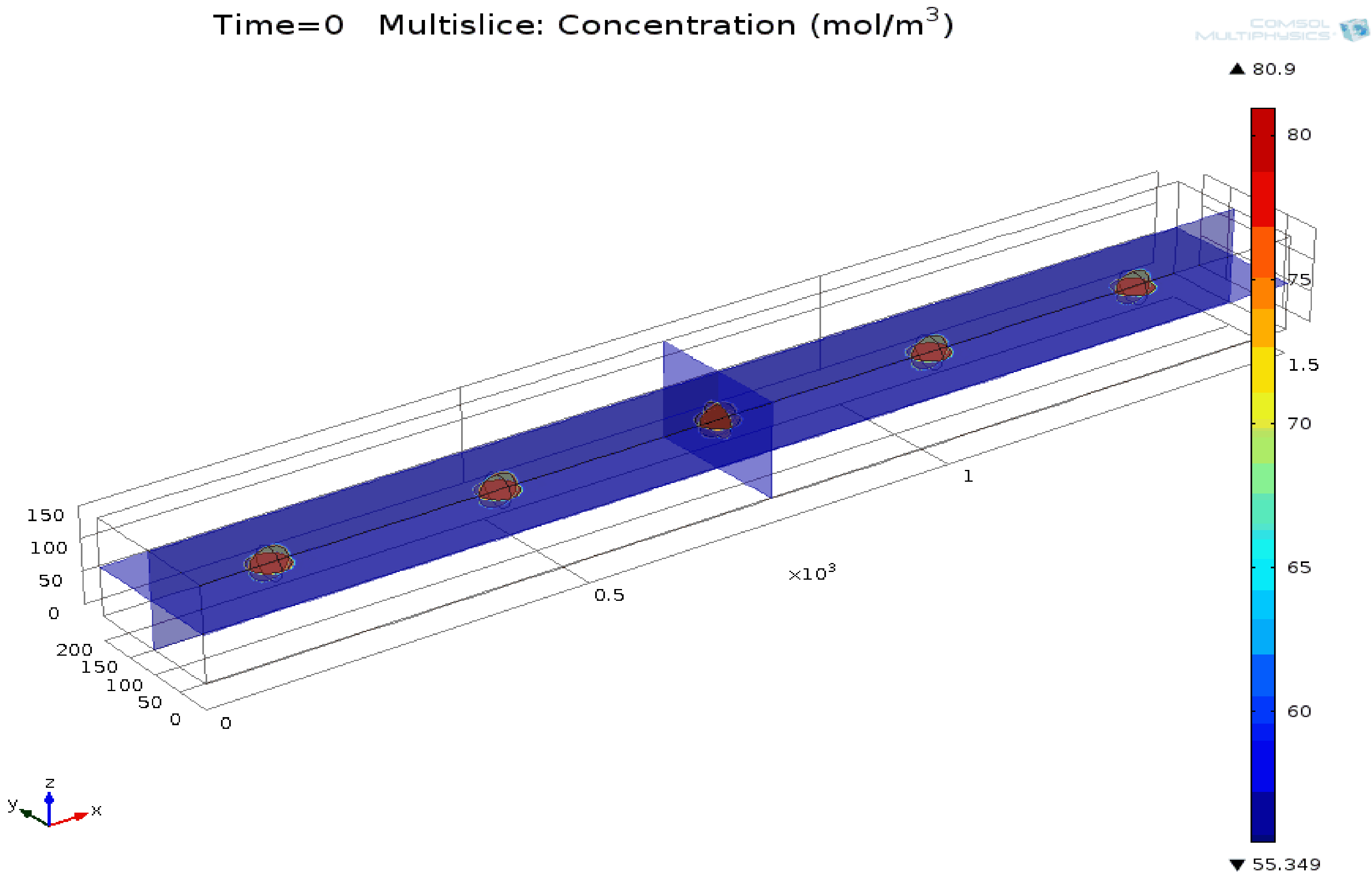
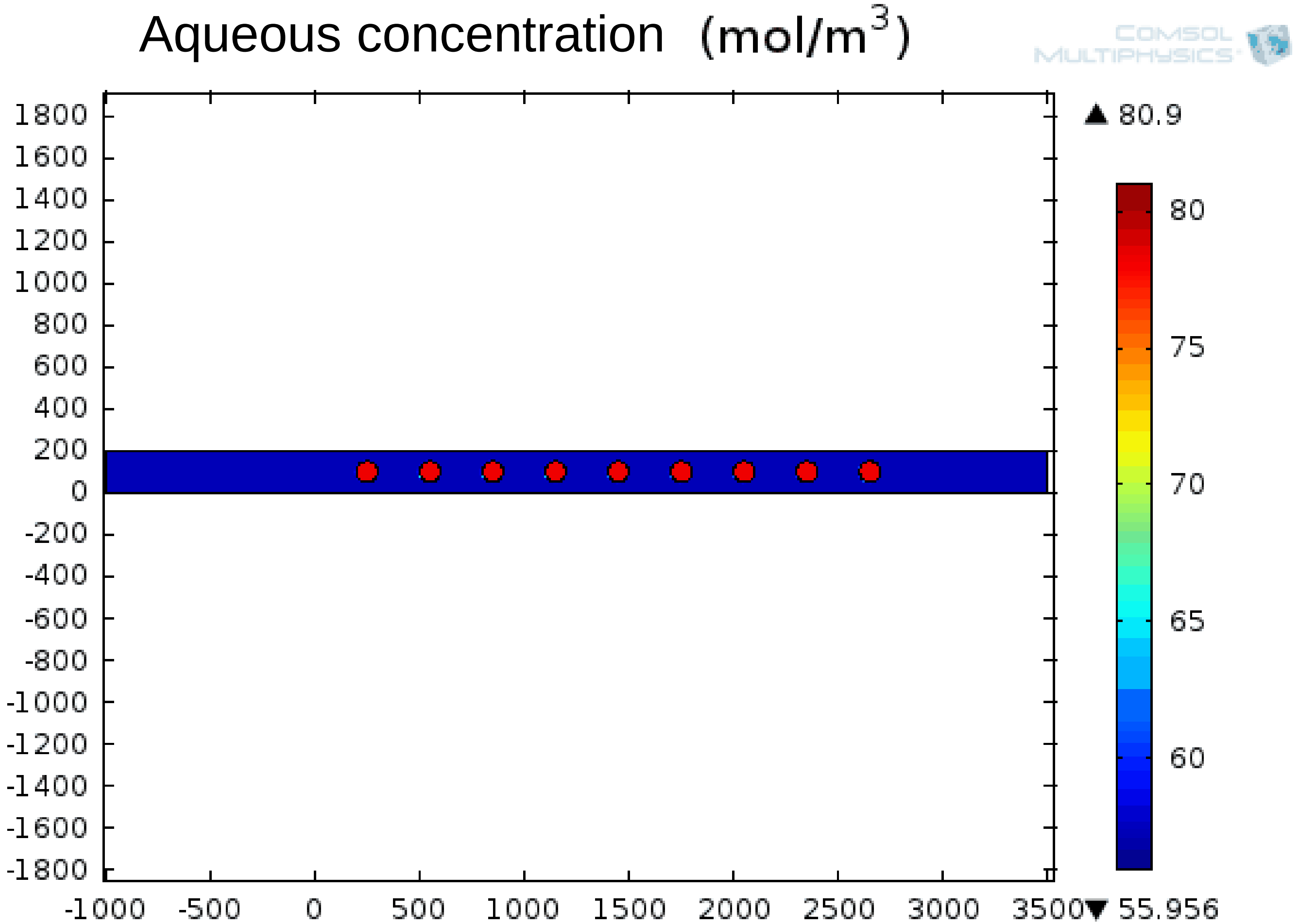
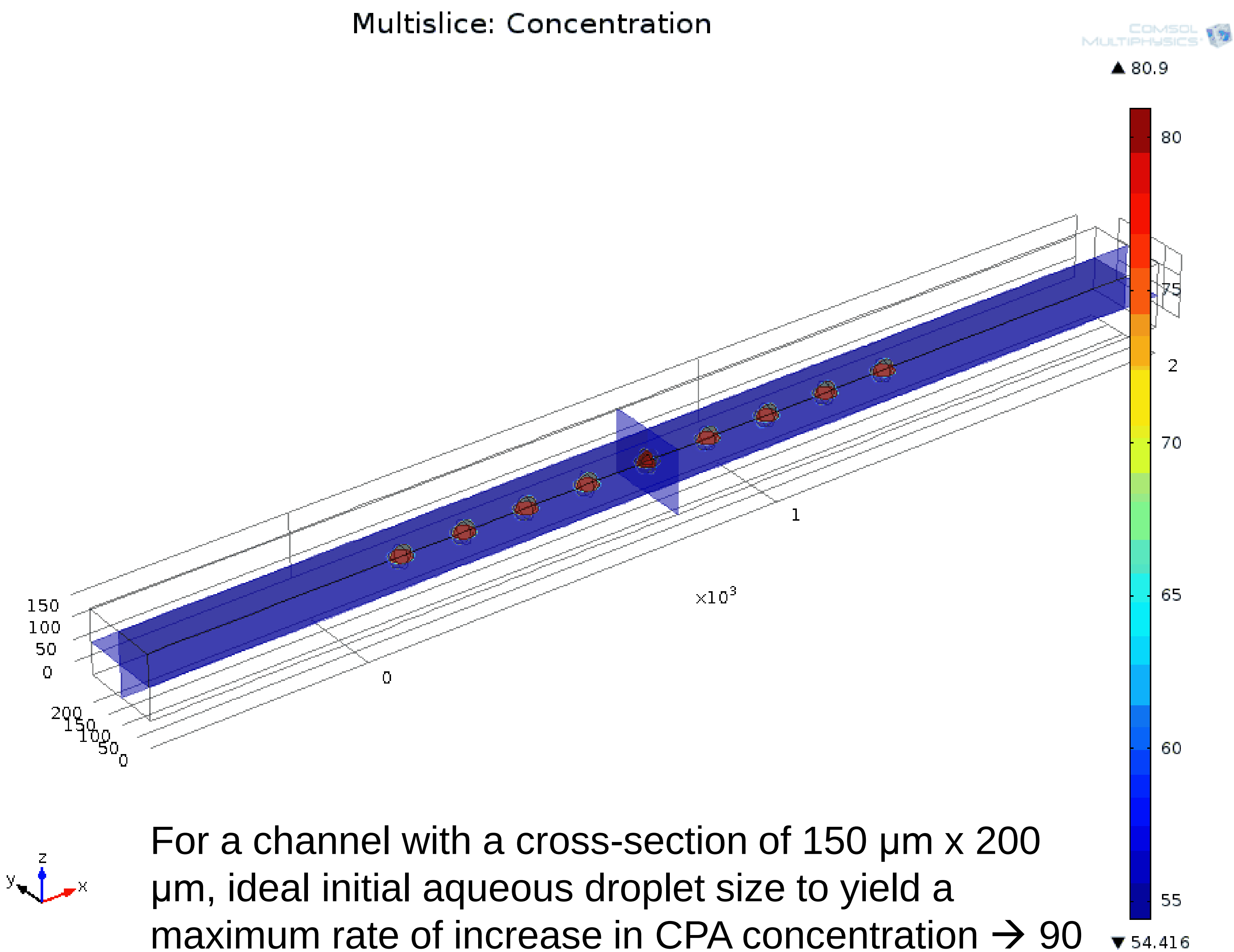
Aqueous concentration profile during flow (rare droplets)

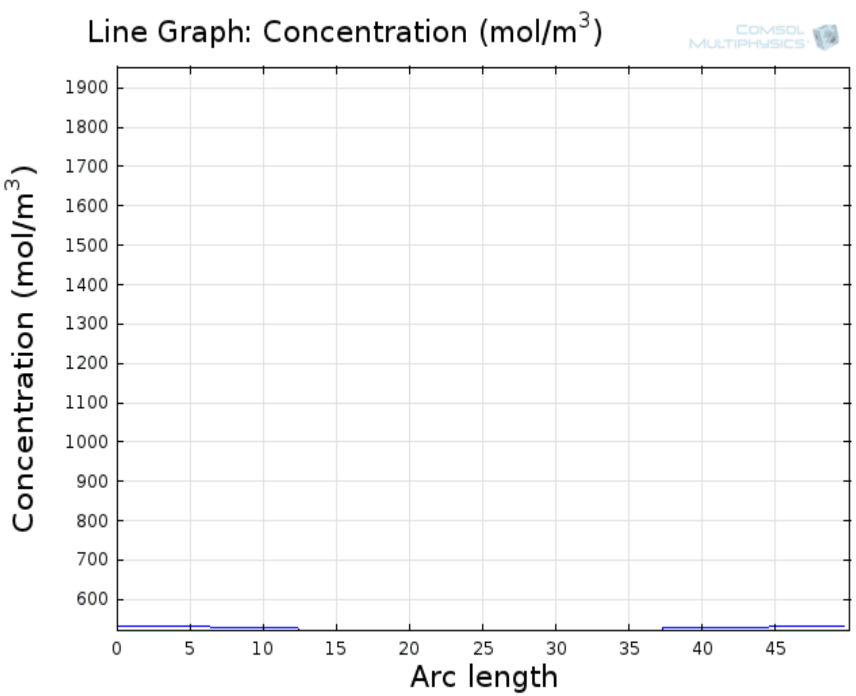


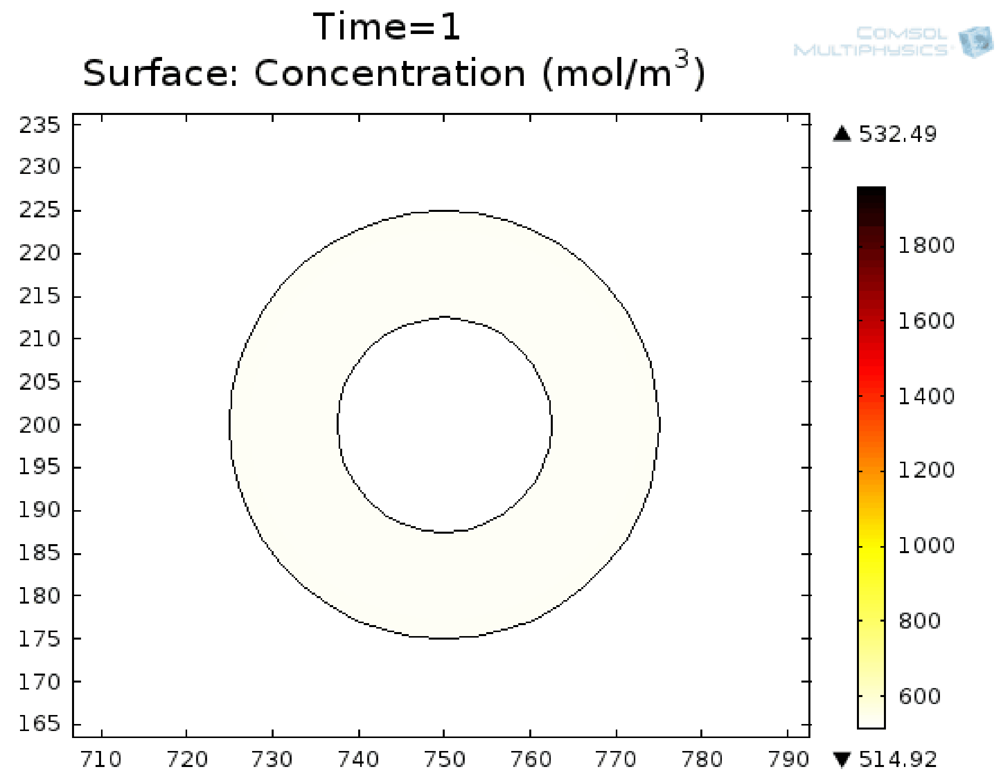

Aqueous concentration (mol/m3)
COMSOL MULTIPHYSICS
▲ 80.9
80
75
70
65
60
▼ 55.956
1800
1600
1400
1200
1000
800
600
400
200
0
-200
-400
-600
-800
-1000
-1200
-1400
-1600
-1800
-1000
-500
0
500
1000
1500
2000
2500
3000
3500



For a channel with a cross-section of 150 µm x 200 µm, ideal initial aqueous droplet size to yield a maximum rate of increase in CPA concentration → 90 µm

Glycerol concentration profile in droplet and cell





Preconcentration with CPAs up to 10x(initial concentration) in <4 min via microfluidic method → Possible

Conclusion

- Preconcentration of cells in dehydrating picoliter aqueous droplets has proven to be a promising method by:
  - Reducing the exposure time of cells to CPAs,
  - Eliminating the multistep CPA loading-diluting procedures, and
  - Avoiding the mechanical and osmotic stresses caused by large concentration differences.

On going:

- Performance tests on the MEMS device without and with various cell lines

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- Hacettepe University Scientific Research Office (BAP)
- Center for Engineering in Medicine, Harvard Medical School, Massachusetts General Hospital, Shriners Hospital for Children, Boston, MA, USA

Problems with freezing

- T>Tg → Crystallization: Intra/extra cellular ice formation during freezing
- CPA at high concetration →Tg ↗
- Intra/extracellular osmotic pressure differences
- Long time exposure to chemicals
- Electrolyte unbalance
- Cell shrinkage due to water removal

All cause damage on cell and its organelles during freezing