

Uniform Amplification of Phage Display Libraries Using Microfluidic Technology

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Abstract

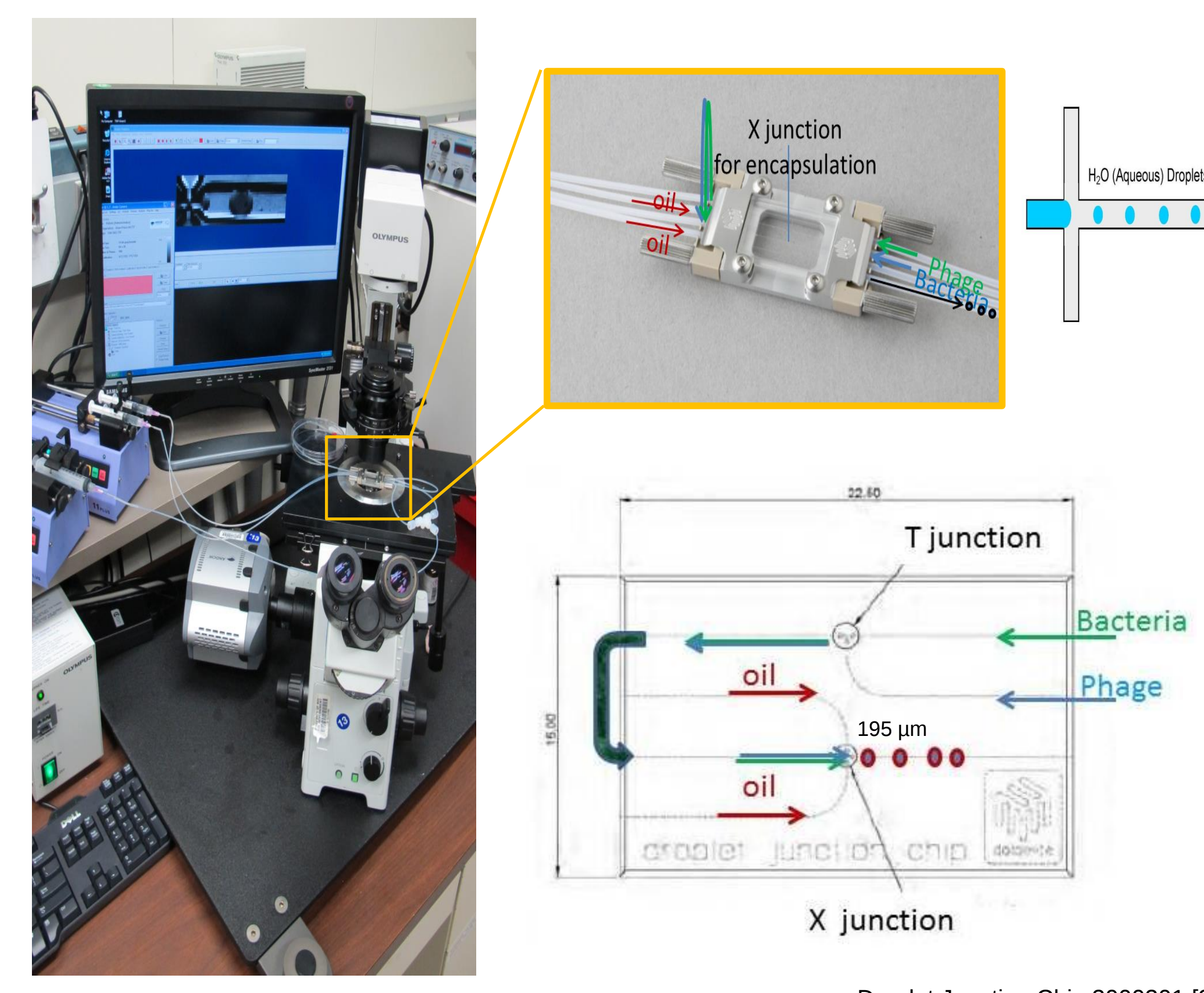
Phage display is a widely-used research and drug discovery technique for creating and screening highly diverse peptide libraries to identify ligands for any target. Affinity selection involves panning of a phage library to enrich for target-binding clones followed by their amplification. Uniform amplification of all members of a library is of utmost importance for all selection experiments. If uniform amplification is not achieved, bias is introduced into the selection that favors faster growing clones regardless of the selection pressure applied. This results in the potential elimination of many biologically relevant binding clones from the screen. Microfluidic flow-focusing technology is used to generate monodisperse droplet-based compartments to encapsulate individual phage clones, resulting in the elimination of competition between phage clones with different growth rates. The elimination of growth-based competition ensures that selection of binding clones is driven only by the binding strength of each clone. The knowledge of these target interactions is an integral part of investigating the mechanistic interactions in cell signaling and is a novel approach for library screening.

Materials and Methods

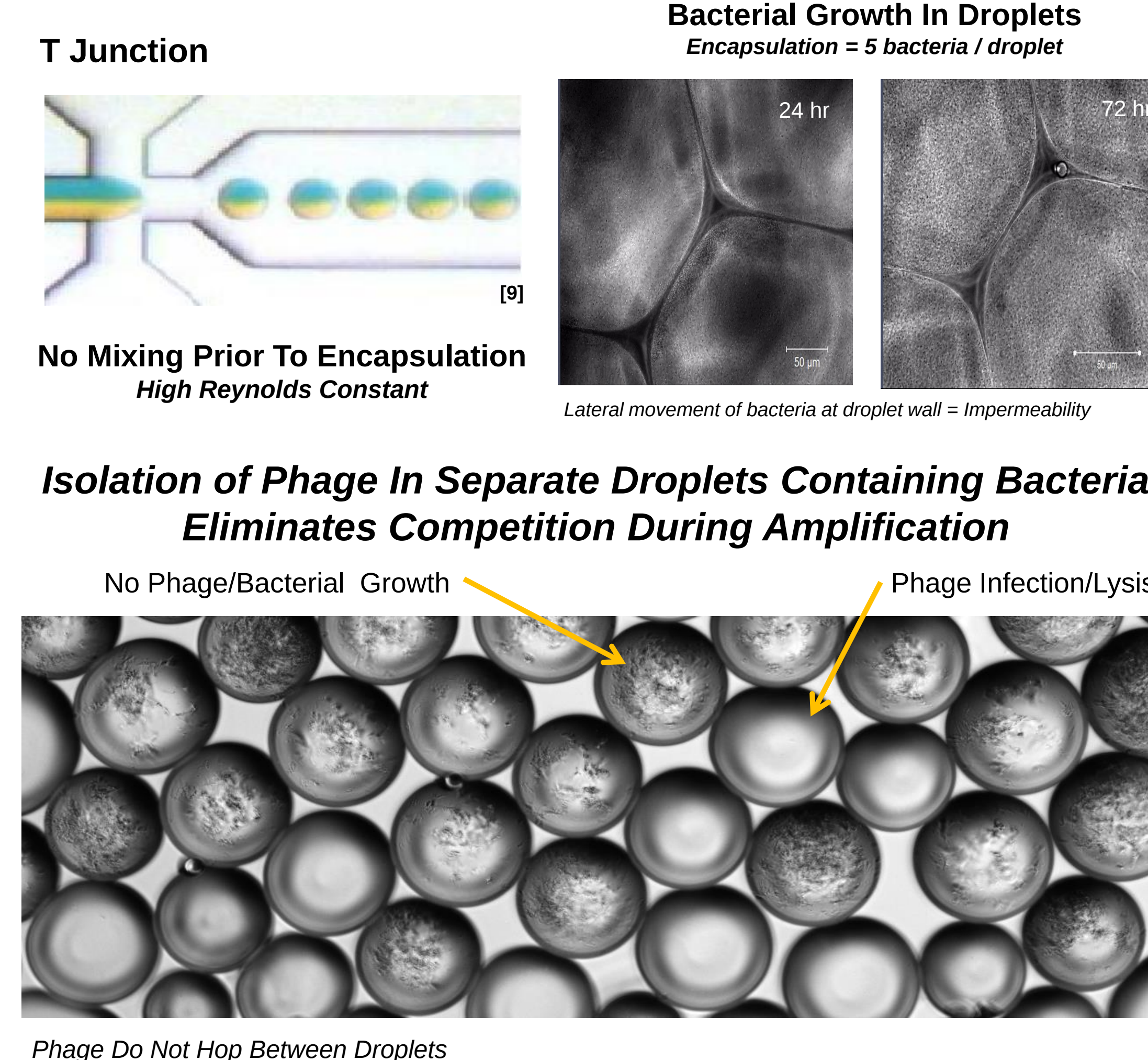
Materials:
Microfluidic Flow-Focusing Device: Droplet Junction Chip (Dolomite DJC 3000301) + Fluorophilic Coating
Single Syringe Pump: Harvard Apparatus Pump 11 Plus (PY270-2208)
Double Syringe Pump: Harvard Apparatus Pump 11 Plus (PY270-2209)
PFPE-PEG-PFPE (RAN Biotechnologies)
HFE-7500 (3M 98-0212-2928-5)
FC-40 (Sigma F9755)
Perfluoro phase: 2% (w/v) PFPE-PEG-PFPE in HFE-7500
BD 10 mL syringe + 21G_{1/2} needle, syringe filter 0.2 µm
Generation of and Culture in Droplets:
Fill channel with perfluoro phase: add 3 mL LB + 150 µL log phase bacteria (syringe 1) + 10 µL 10¹⁰ pfu/mL T7 phage in 3 mL 1x PBST (syringe 2) = 1 phage/droplet and ~5 bacteria/droplet.
240 µl/min oil / 60 µl/min aqueous: R1=95 µm, R2=195 µm, R3=95 µm
Collection in 3 cm petri dish with 500 µL HFE-7500 inside 14 cm petri dish with water (provides humid environment for droplets and minimizes accumulation of static electricity on dry plastic dishes). Place PE tubing from outlet of channel into petri dish on top of perfluorocarbon layer.
Amplification of phage (4-5 hr), harvest phage from droplets via destabilizing solution: 0.5% (w/v) Krytox in HFE-7100
Imaging of Droplets:
ANDOR high speed camera (500 Hz) with Image J ROI

Microfluidic Flow-Focusing Device

NIEHS
2013



Bacteria & Phage Encapsulation



Introduction

Selection from phage display libraries is driven by two **independent** selection processes: (1) the **panning** step enriches clones that bind to the desired target or any other physical moieties present during the panning step (2) the **amplification** step (infection of bacteria by a single phage particle to create multiple copies of genetically identical phage) enriches clones that have a growth advantage/amplify faster during any of the amplification steps. As a result, fast growing weak or nonspecific binders may overgrow and ultimately be 'selected' over more desirable slowly growing strong binders. Amplification induced loss of diversity severely hinders the identification of ligands from any display system. To eliminate this bias, individual phage must be separated into different growth chambers so they cannot compete for bacterial hosts.

Results

True uniform amplification of phage libraries can be achieved in monodisperse droplets formed in a microfluidics channel

- ♦ **Droplets isolate phage clones from one another**
Impermeability: bacteria & phage cannot jump b/w droplets
- ♦ **Supply each clone with an equal number of bacteria**
Monodispersity: each droplet is identical in size
- ♦ **Allow amplification to go to completion**
Droplet Stability: allowing all bacteria to be infected by phage

Droplet amplification of phage libraries eliminates the competition between phage clones that have different growth characteristics

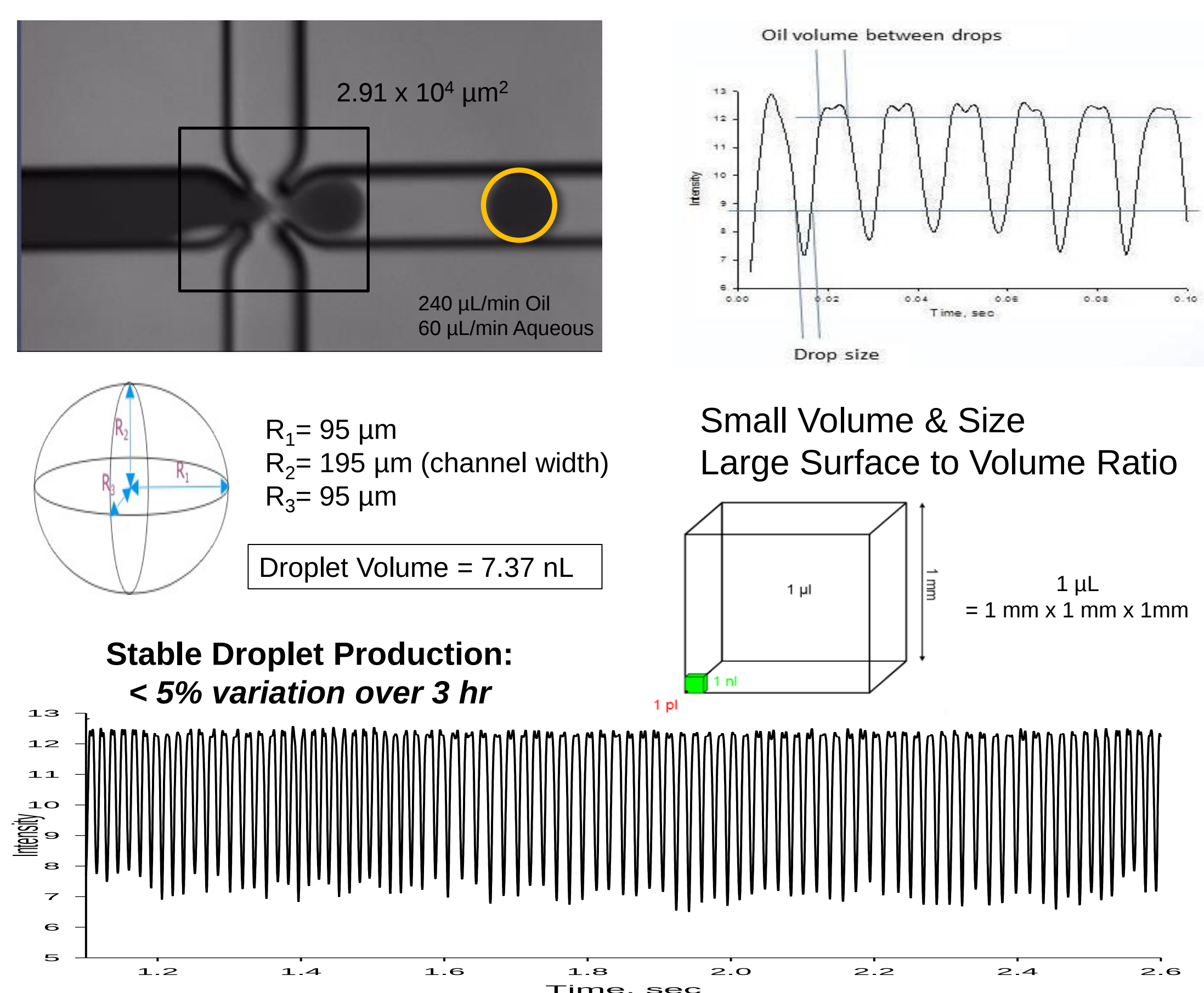
The elimination of growth-based competition ensures that selection of binding clones is driven only by the binding strength of each clone.

Elimination of undesired competition between different phage clones enable:

- * Selection of wider repertoire of target-binding phage independent of their relative rates of replication
- * Identification of rare ligands
- * Prediction of target affinity based on abundance of clones

Droplet Monodispersity

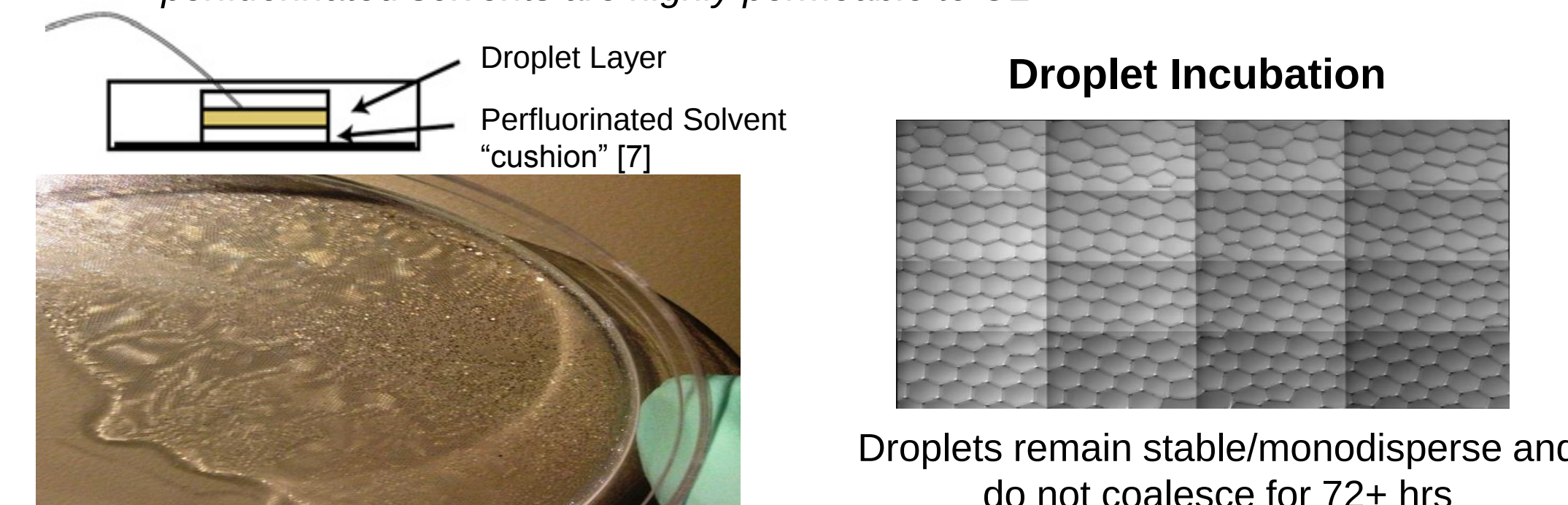
Encapsulate Nanoliter Volumes of Aqueous Solutions



Droplet Stability

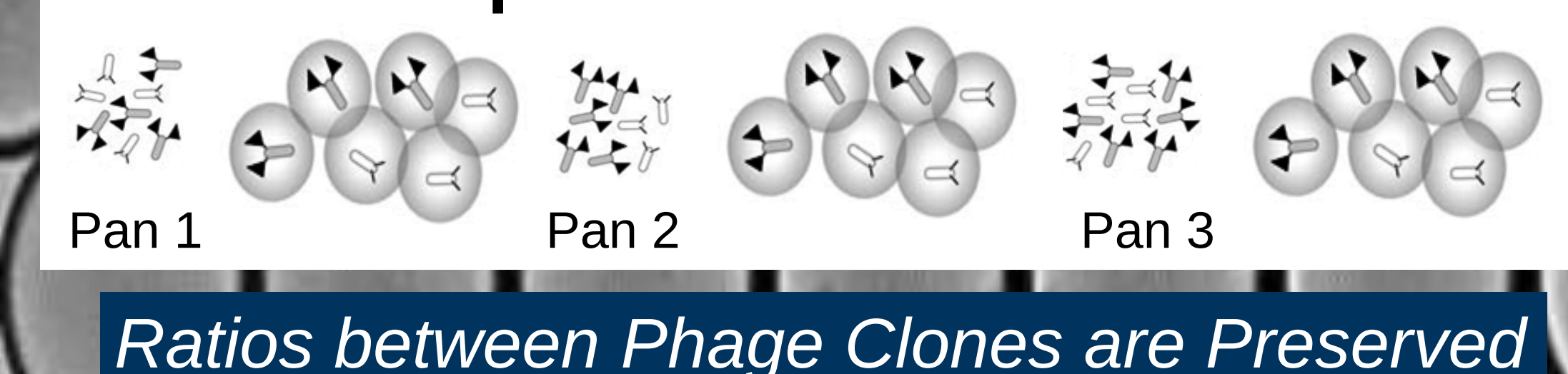
Water-In-Oil Emulsion:

- Stabilized by non-ionic Fluorosurfactant PFPE-PEG-PFPE
 - Make droplets compatible with biological molecules and cells
- Phage must remain in separate droplets with bacteria hosts for the duration of amplification (4-5 hr). *No coalescence of droplets during this incubation period*
- Each droplet must contain a clonal population of phage. *Dilution*
- During amplification of phage, bacterial host require oxygen and nutrients *perfluorinated solvents are highly permeable to O2*



The Solution:

Uniform Amplification Between ERα Pans



Applications of Droplet-Based Microfluidics

Benefits:

- Creates discrete monodisperse isolated environments
- Sample volume significantly reduced *lower costs*
- Manipulation at kilohertz speeds (10,000 droplets/sec) *shorter time to assay large libraries*
- Compartmentalization *higher sensitivity*
- Monodispersity/Size Consistency *accurate & repeatable experiments*
- Droplet Stability/No Cross Contamination *monitor biological process over extended period of time*

Uniform Amplification of Display System Phage, Ribosome, RNA, DNA

Ultrahigh-Throughput Biological Screens

Compartmentalized Chemistry Mini Chemical Reactors

DNA Pyrosequencing/PCR Amplification/ RT-PCR

References

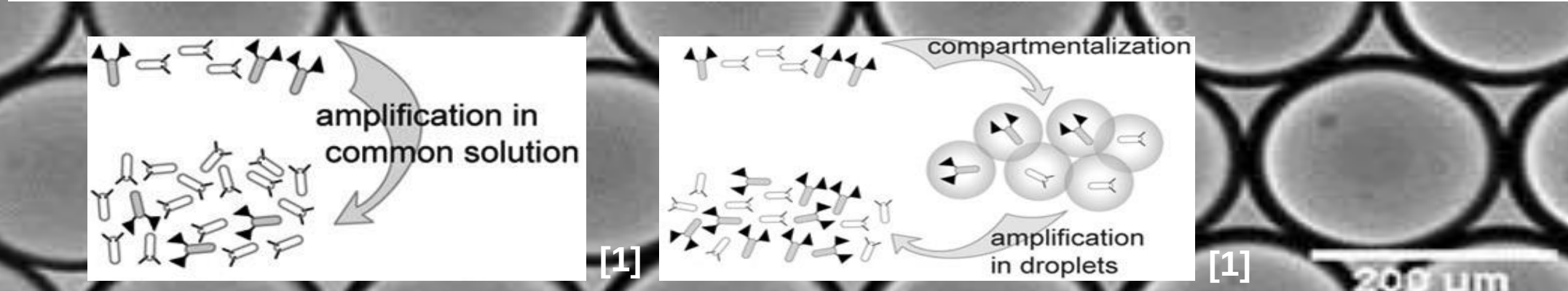
1. Derda, R.; Tang, S.K.; Li, S.C.; Ng, S.; Matochko, W.; Jafari, M.R. *Molecules* 2011; 16: 1776-1803.
2. Derda, R.; Musah, S.; Omer, B.P.; Klim, J.R.; Li, L.; Kiessling, L.L. *J. Am. Chem. Soc.* 2010; 132:1289-1295.
3. Derda, R.; Tang, S.K.; Y. and Whitesides, George M. *Angew. Chem., Int. Ed.* 2010; 49: 5301-5304.
4. Hoen, P.A.; Jirka, S.M.; Ten Broeke, B.R.; Schultes, E.A.; Aguilera, B.; Pang, K.H.; Heemskerk, H.; Aartsma-Rus, A.; van Ommen, G.J.; den Dunnen, J.T. *Anal. Biochem.* 2012; 421(2):622-31.
5. Kuzmicheva, G.A.; Jayanna, P.K.; Sorokulova, I.B.; Petrenko, V.A. *Protein Eng Des Sel.* 2009; 22(1):9-18.
6. Lauren, R.H.; Krumpke, Toshiyuki Mori. *Int. J. Pept. Res. Ther.* 2006; 12(1): 79-91.
7. Matochko, W.L.; Ng, S.; Jafari, M.R.; Romanuk, J.; Tang, S.K.; Derda, R. *Methods.* 2012; 58(1):18-27.
8. Wilson, D.R.; Fintay, B.B. *Can. J. Microbiol.* 1998; 44(4):313-29.
9. Dolomite Centre Ltd. *Droplet Junction Chips*. DJC 3000301

Objective

Uniform amplification cannot be achieved when multiple phage having different growth rates compete with each other in a common solution.

→ **Use Microfluidic Technology to generate monodisperse droplet-based compartments to encapsulate individual phage clones from a mixture of clones possessing different growth characteristics.**

The ratio between clones can be preserved if, and only if, the clones are isolated from each other and all compartments are the same size.



The Problem:

Panning vs. Rate of Amplification

