

A highly efficient bacteriophage screening method using water-in-oil droplet technology

○Miu Hoshino^{1,2}, Yuri Ota^{2,3}, Tetsushi Suyama², Satoshi Tsuneda⁴, Naohiro Noda^{1,2,4}

¹University of Tokyo, Grad. Sch. Frontier Science, ²AIST BMRI, ³On-chip Biotechnologies Co. Ltd., ⁴Waseda University, Grad. Sch. Advanced Science and Engineering



Contact: Miu Hoshino (miu-hoshino@aist.go.jp)
Naohiro Noda (noda-naohiro@aist.go.jp)

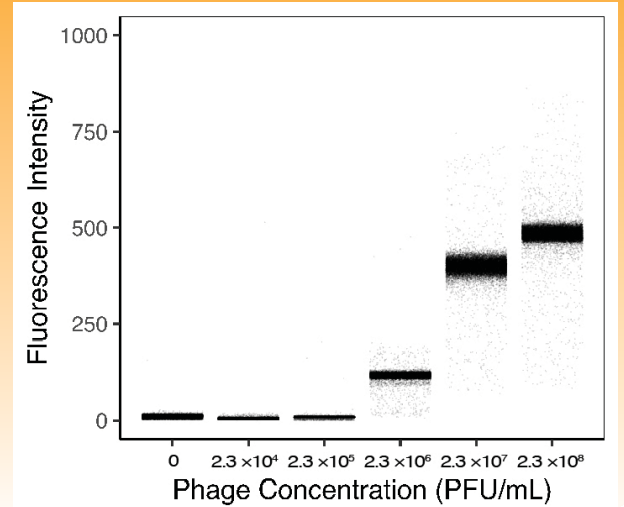
Background and Outline

- Water-in-oil droplets are miniscule drops of water dispersed in oil. Each droplet acts as an individual compartment.
- The high throughput of droplet technology is extremely suitable for use in screening experiments.
- Co-cultivation of T2 phage and host bacteria within droplets resulted in phage propagation within droplets. Progeny phage were detained in their respective droplets.
- Addition of YOYO-1 into droplets enabled differentiation of droplets with phage via droplet fluorescence intensity.
- Isolation of droplets enabled isolation of phage within.

Phage quantity and fluorescence

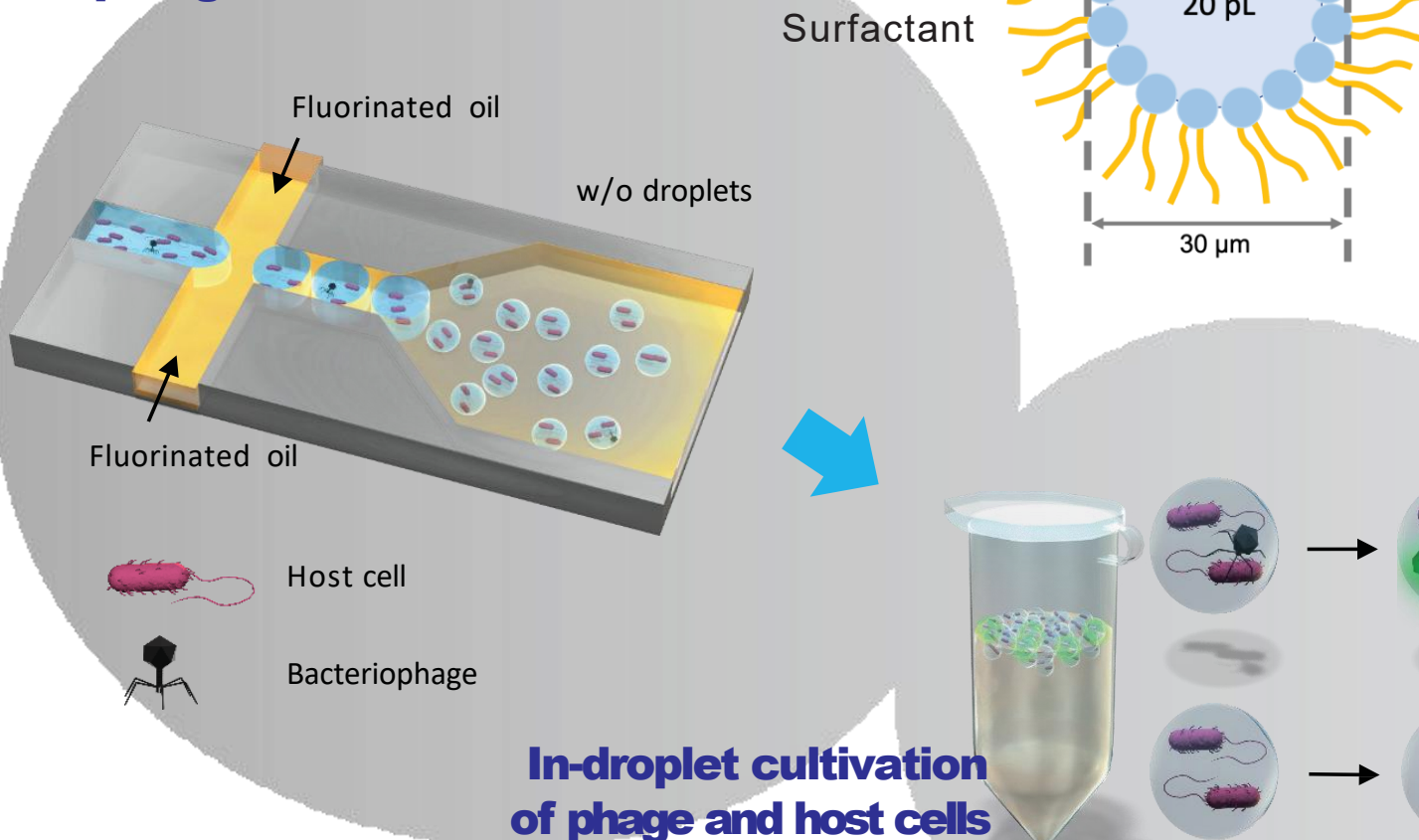
Fluorescence intensities of droplets containing YOYO-1 and various concentrations of T2 phage were measured.

Fluorescence intensity reflects quantity of phage within droplets



The finalized flow for phage screening

Droplet generation



YOYO-1

- Cell membrane impermeant
- Nucleic acid intercalator



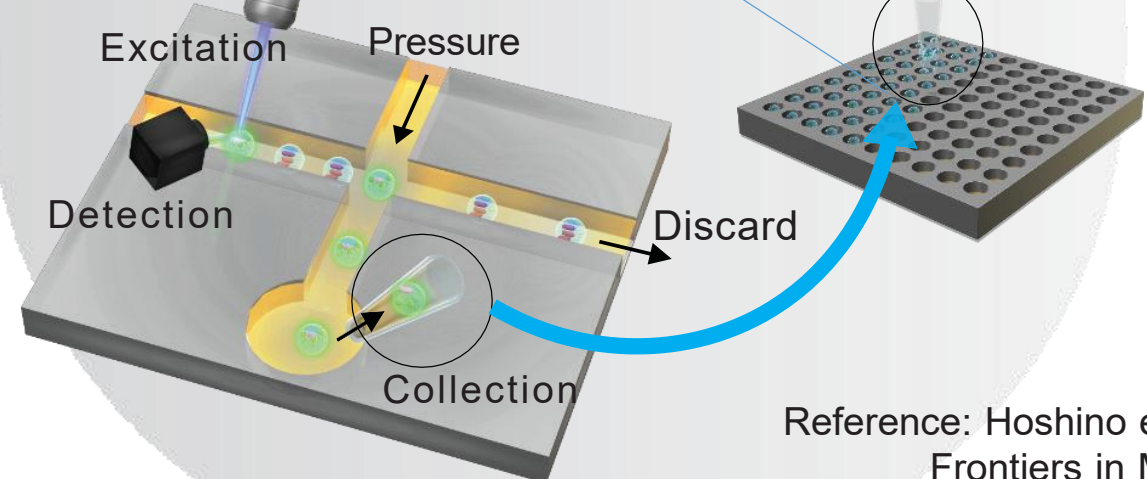
Phage recovery from single droplets

Droplets (with high AND low fluorescence intensities) were isolated into 96-well plates and subjected to rupturing and up-scale cultivation of phage.

- 35.7% phage recovery rate from droplets with high fluorescence intensities**
- 0% phage recovery rate from droplets with low fluorescence intensities**

*Existence of phage was examined via spot assay

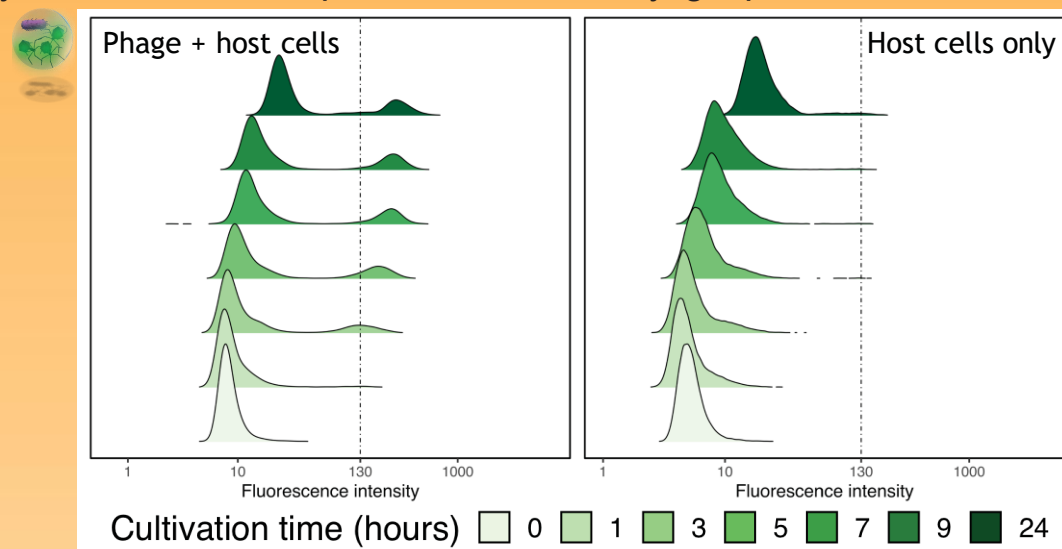
Isolation and rupturing of individual droplets followed by upscaled cultivation of phage



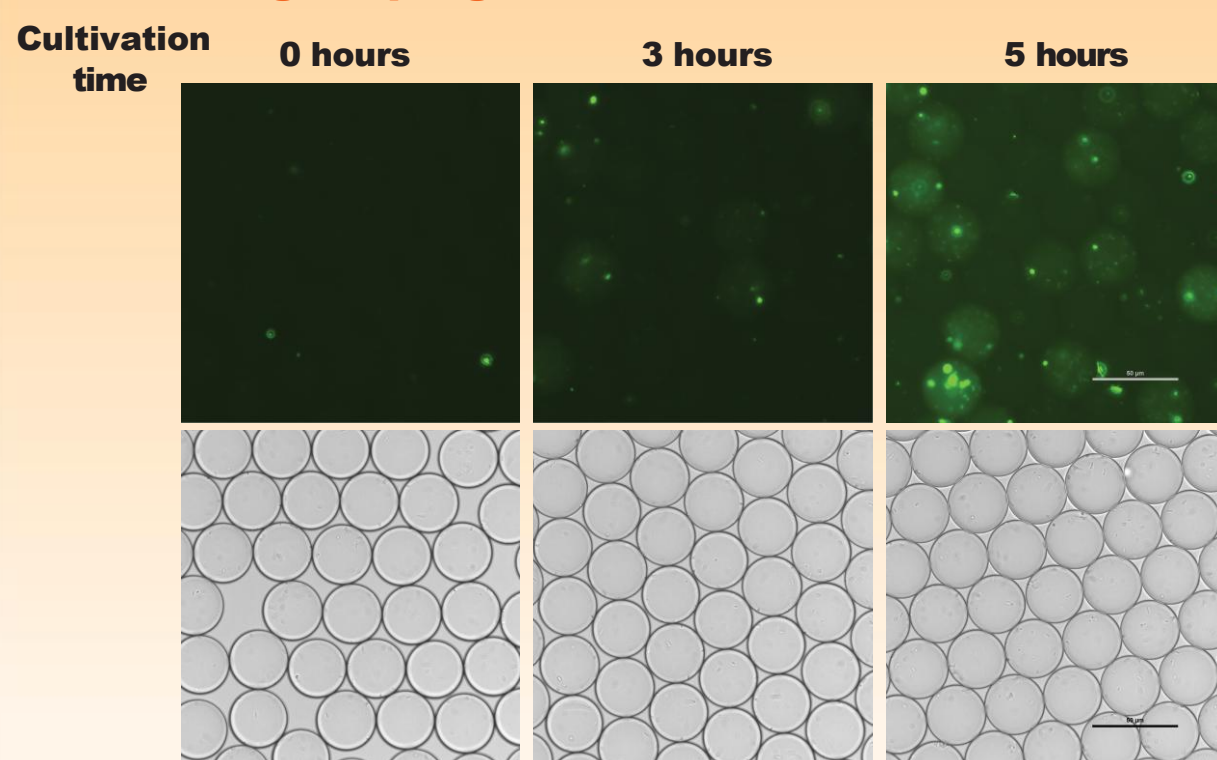
Reference: Hoshino et al., (2023) Frontiers in Microbiology

Transition of droplet fluorescence intensity distribution during cultivation

Droplets containing phage, host cells and YOYO-1 (left), and droplets containing host cells and YOYO-1 (right) were generated. Fluorescence intensities of roughly 40,000 droplets were measured every few hours and plotted as density graphs.



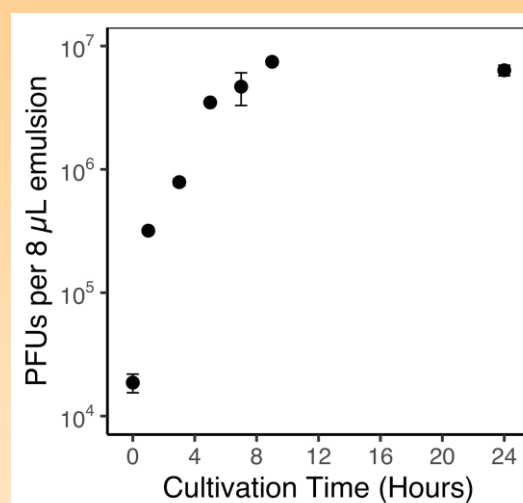
- Fluorescence intensities started increasing after only 3 hours of cultivation**
- Proportion of non-fluorescent droplets can be used to calculate original phage titer**



Droplets observed over time

Growth of in-droplet phage titer during cultivation

A fixed volume of emulsion was sampled from the droplets generated with phage, host cells and YOYO-1 every few hours. These were ruptured and the contents used to perform plaque assays.



- Phage propagated rapidly in the first few hours (~5hours) after segregation into droplets**
- Phage stopped propagating after 9 hours**

Conclusions and future directions

- This method can be used not only for isolation of phage, but also for determining titer of phage suspensions
- Phage recovery rate was 35.7% from fluorescent droplets, but none were recovered from non-fluorescent droplets
- Modifications may be necessary for screening non-lytic phage
- This method requires no cultivation on agar, which should simplify acquisition of phages infecting hard-to-culture hosts.

Acknowledgements •This work was supported by JST SPRING, Grant Number JPMJSP2108.

•This work is based on results obtained from project, JPNP20011, commissioned by the New Energy and Industrial Technology Development Organization (NEDO).

•This work was supported by Cabinet Office, Government of Japan, Cross-ministerial Strategic Innovation Promotion Program (SIP), "Technologies for Smart Bio-industry and Agriculture" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).