Droplet-on-Demand Platform for Biochemical Screening & Drug Discovery

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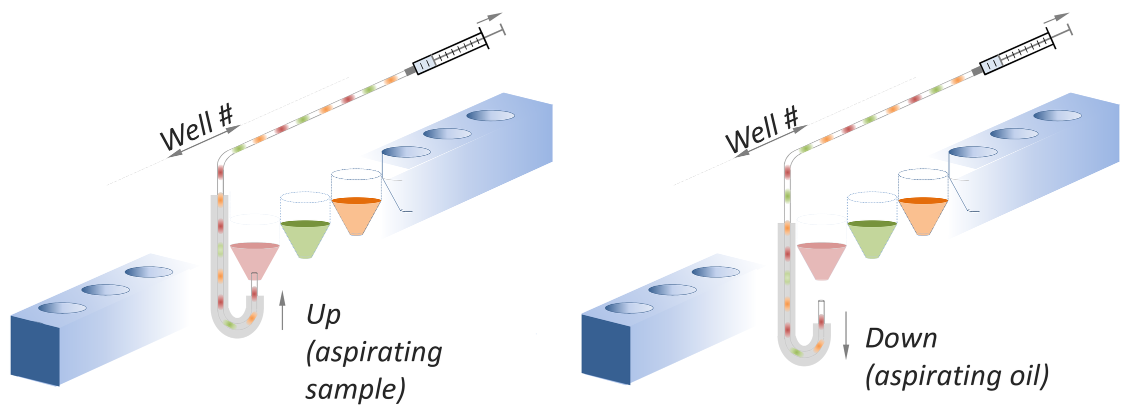
Aim

To demonstrate droplet on demand applications towards study of biological entities encapsulated in nanoliter droplets. Interfacing a droplet on demand platform with microfluidic chips allows for merging and dilution of droplets. This feature is applied to encapsulate yeast cells (*S. cerevisiae*) and multi-cellular organisms (*C. elegans*).

Droplet on Demand Technology

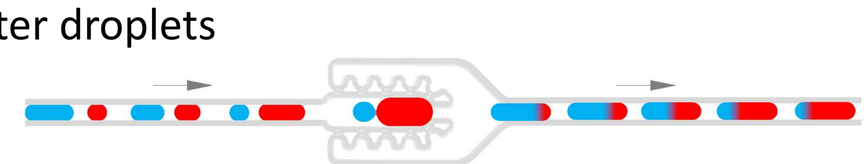
Droplet Creation Mechanism



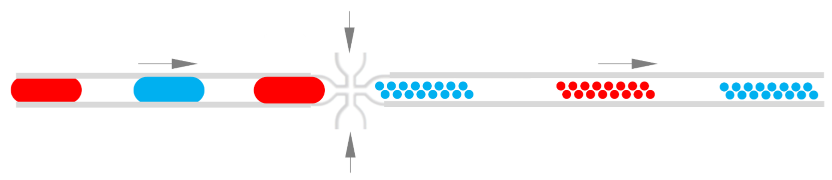
A constant aspiration is set up using a syringe pump. The sampling tube is programmed to move between aqueous and fluorocabon fluids, thereby the creating plugs of the two immiscible fluids.

Droplet Merging and Splitting Mechanism

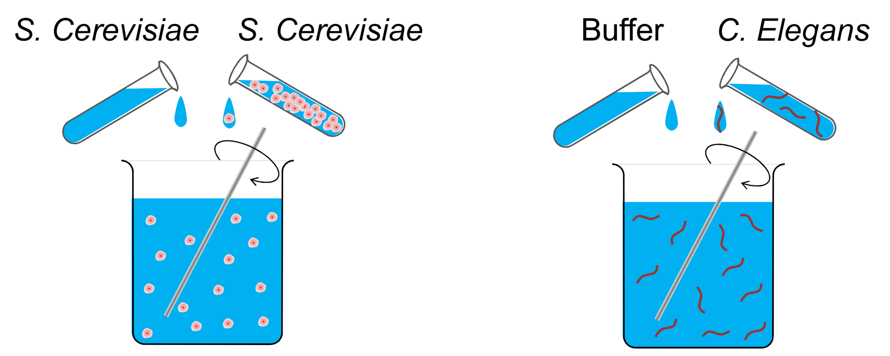
- Merging successive pairs after creating a sequence of nanoliter droplets



- Splitting each nanoliter droplet into picoliter droplets

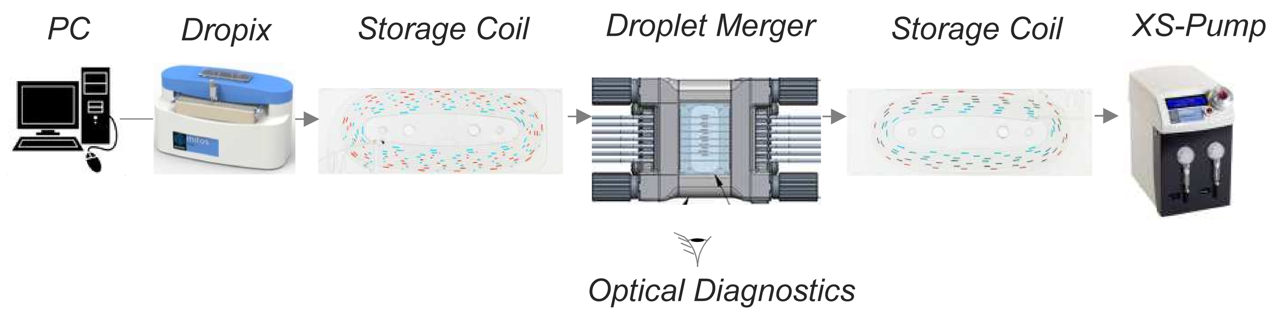


Method



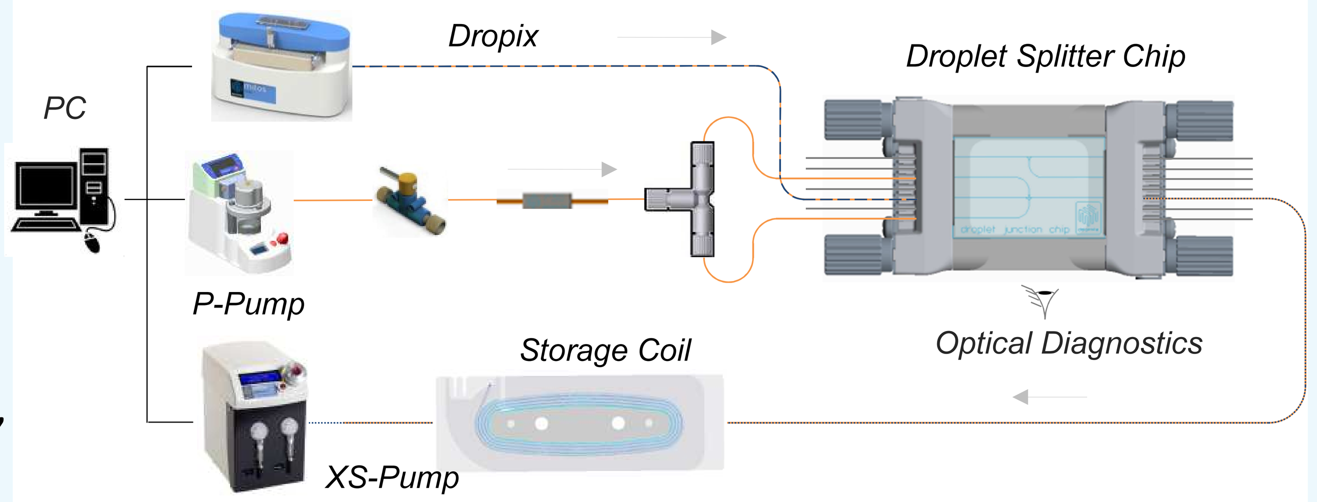
A culture of *S. Cerevisiae* cells is mixed in with cell buffer to make a resulting solution of 500 µL. In a separate test, 5 day old *C. Elegans* embryos are mixed with buffer to make a dilute solution. 50 µL of each is pipetted into the sample strip of the Dropix.

Test Setup 1 – Droplet Merging



- Setup consists of 1 syringe pump, 1 merging chip, 2 storage coils, and 1 Dropix. Syringe pump creates a suction driven flow.
- Flow rates range from 1 µL/min to 20 µL/min
- Merging chip has channel depth of 200 µm
- Merged droplet volume ranges from 50 to 200 nL

Test Setup 2 – Droplet Splitting

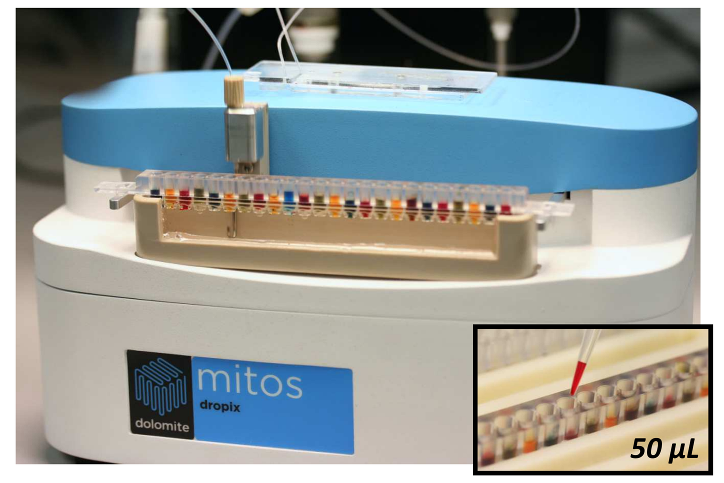


- Setup consists of 1 syringe pump, 1 droplet splitter chip, 2 storage coils, and 1 Dropix.
- Chip has a depth of 100 µm. Flow rates 1 – 20 µL/min.

Test Apparatus

Specifications

- Generates droplets of up to 24 different samples
- Droplet production frequency of 5Hz.
- Droplet size range from 50 nL to 50 µL.
- Stores up to 1000 droplets.



Mitos Dropix – Droplet on demand platform. Each of the 24 fluid reservoirs holds up to 50 µL.

The Mitos Dropix® system enables

- Automated screening experiments (sample/screen pairing)
- Dose-response testing (sample/diluent pairing to deliver to screen)
- Concentration/Stoichiometric testing (variation of mix ratio)
- Combinatorial chemistry (A_nB_n reagent pairings)

Results

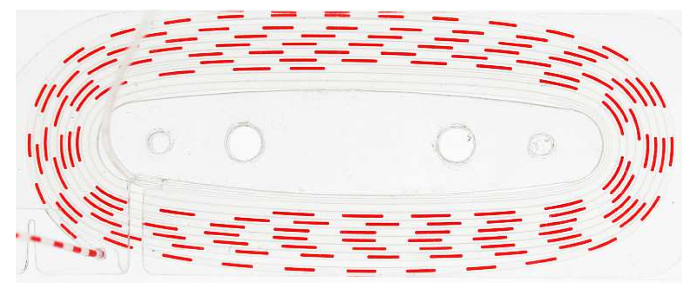
Sequence Generation

Droplet sequence of various sized nanoliter droplets.

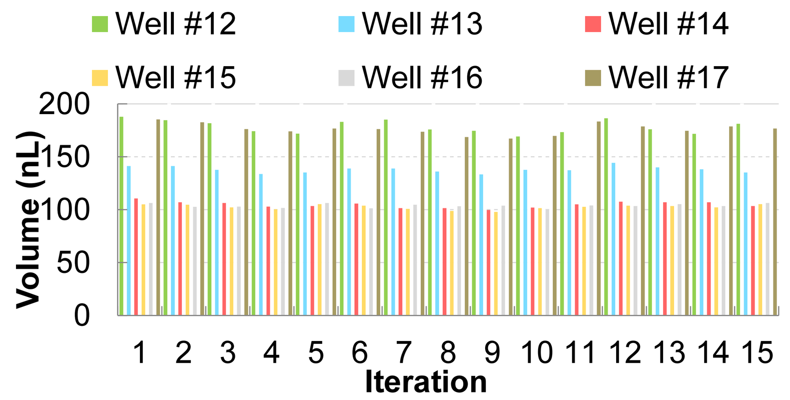
Droplet Merging

200 nL droplets. 100 step concentration gradient created from merging two liquids.

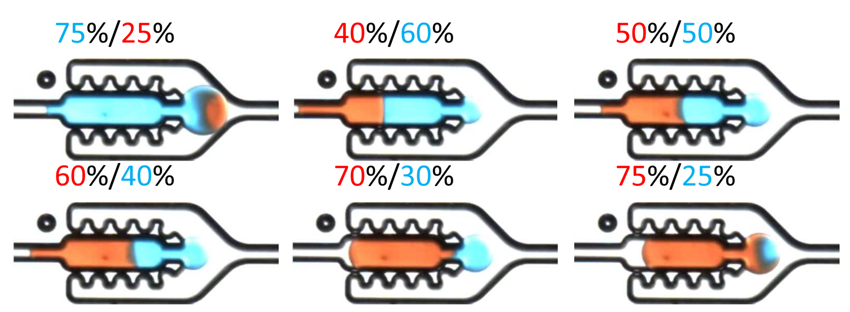
Droplet Splitting



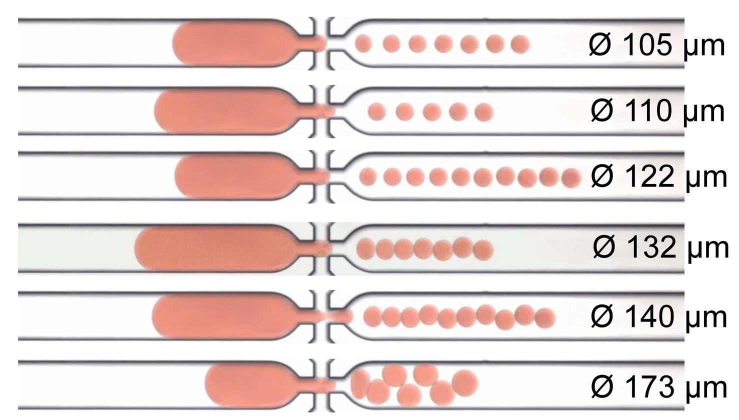
200 nL droplet sequence before splitting.



Multiple iterations of a 6 droplet sequence. Droplet volumes are 200, 150, 100, 100, 100, 200 nL.

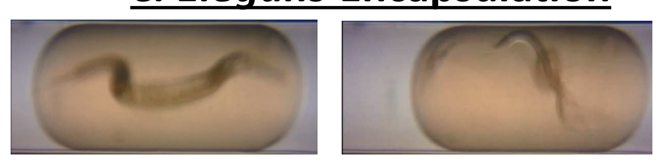


Red & blue droplets merging in varying proportions to create a concentration gradient.



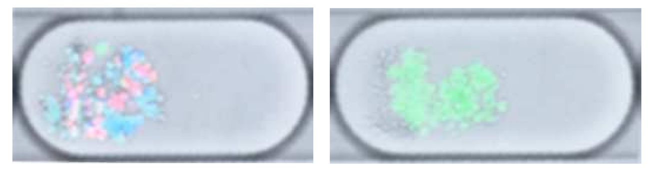
A nanoliter droplet split into multiple picoliter droplets.

C. Elegans Encapsulation



C. Elegans cells encapsulated in nanoliter droplets.

S. Cerevisiae Encapsulation



S. Cerevisiae cells encapsulated in nanoliter droplets

Conclusion

- The method for generating specific sequences of droplets is previously described [1]. Now several modes are demonstrated to enable a wider range of applications.
- Sequences of droplets can be generated from 24 wells.
- Droplets can be merged pairwise.
- Nanoliter droplets can be split into picoliter droplets.
- Our system enables combinatorial screening in nanolitre volumes and we have demonstrated its application to drug screening whether in vitro or in vivo.
- Encapsulated *S. Cerevisiae* cells showed good growth for all cell strains overnight.
- *C. elegans* were also encapsulated and were stable in droplets overnight.

Future Outlook

- Merging yeast cells with different drugs has the potential to show different growth patterns of pooled yeast strains.
- This will be a valuable tool for drug selectivity studies [3]. C. Elegans can be encapsulated using our system, and merging encapsulated organisms with drugs could be used to study effects on neural mechanism.

References

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[2] Niu et al. A microdroplet dilutor for high-throughput screening. *Nat. Chem*., 3, 437-442, **2011**.

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